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(54) Title: LIPOPOLYSACCHARIDE BINDING PROTEIN DERIVATIVES

(57) Abstract

Disclosed are novel biologically active lipopolysaccharide binding protein (LBP) derivatives including LBP derivative hybrid proteins which are characterized by the ability to bind to and neutralize LPS and which lack the CD14-mediated immunostimulatory properties of holo-LBP.

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LIPOPOLYSACCHARIDE BINDING PROTEIN DERIVATIVES

This is a continuation-in-part of U.S. Patent Application Serial No. 08/079,510 filed June 17, 1993.

BACKGROUND OF THE INVENTION

The present invention relates generally to proteins useful for the treatment of gram-negative bacterial infections and specifically to the neutralization of the effects of lipopolysaccharide (LPS) which is also known as endotoxin. LPS is a major component of the outer membrane of gram-negative bacteria and consists of serotype-specific O-side chain polysaccharides linked to a conserved region of core oligosaccharide and lipid A. LPS is an important mediator in the pathogenesis of septic shock and is one of the major causes of death in intensive-care units in the United States. It has been observed that exposure to LPS during sepsis stimulates an immune response in monocytes and macrophages that results in a toxic cascade resulting in the production of tumor necrosis factor (TNF) and other proinflammatory cytokines. Morrison and Ulevitch, *Am. J. Pathol.*, 93:527 (1978). Endothelial damage in sepsis probably results from persistent and repetitive inflammatory insults. Bone, *Annals Int. Med. 115:*457 (1991).

LPS-binding proteins have been identified in various mammalian tissues. Among the most extensively studied of the LPS-binding proteins is bactericidal/permeability-increasing protein (BPI), a basic protein found in the azurophilic granules of polymorphonuclear leukocytes. Human BPI protein has been isolated from polymorphonuclear neutrophils (PMNs) by acid extraction combined with either ion exchange

chromatography or *E. coli* affinity chromatography. Weiss et al., *J. Biol. Chem.*, *253*:2664 (1978); Mannion et al., *J. Immunol. 142*:2807 (1989).

The holo-BPI protein isolated from human PMNs has potent bactericidal activity against a broad spectrum of gram-negative bacteria. This antibacterial activity appears to be associated with the amino terminal region (i.e. amino acid residues 1-199) of the isolated human holo-BPI protein. In contrast, the C-terminal region (i.e. amino acid residues 200-456) of the isolated holo-BPI protein displays only slightly detectable anti-bacterial activity. Ooi et al., *J. Exp. Med.*, *174*:649 (1991). Human DNA encoding BPI has been cloned and the amino acid sequence of the encoded protein has been elucidated. Gray et al., *J. Biol. Chem.*, *264*:9505 (1989). Amino-terminal fragments of BPI include a natural 25 Kd fragment and a recombinant 23 Kd, 199 amino acid residue amino-terminal fragment of the human BPI holoprotein referred to as $rBPI_{23}$. See, Gazzano-Santoro et al., *Infect. Immun. 60*:4754-4761 (1992). In that publication, an expression vector was used as a source of DNA encoding a recombinant expression product ($rBPI_{23}$) having the 31-residue signal sequence and the first 199 amino acids of the N-terminus of the mature human BPI, as set out in SEQ ID NOS: 11 and 12 taken from Gray et al., *supra*, except that valine at position 151 is specified by GTG rather than GTC and residue 185 is glutamic acid (specified by GAG) rather than lysine (specified by AAG). Recombinant holoprotein referred to herein as rBPI has also been produced having the sequence set out in SEQ ID NOS: 11 and 12 taken from Gray et al., *supra*, with the exceptions noted for $rBPI_{23}$. See also, Elsbach et al., U.S. Patent No. 5,198,541 the disclosure of which is hereby incorporated by reference. In addition to its bactericidal effects, BPI has been shown to neutralize the toxic and cytokine-inducing effects of LPS to which it binds.

Lipopolysaccharide binding protein (LBP) is a 60 kD glycoprotein synthesized in the liver which shows significant structural homology with BPI. Schumann et al. disclose the amino acid sequences and encoding cDNA of both human and rabbit LBP. Like BPI, LBP has a binding site for lipid A and binds to the LPS from rough (R-) and smooth (S-) form bacteria. Unlike BPI, LBP does not possess significant bactericidal activity, and it enhances (rather than inhibits) LPS-induced TNF production. Schumann et al., *Science*, *249*:1429 (1990). Thus, in contrast to BPI, LBP has been recognized as an immunostimulatory molecule. See, e.g., Seilhamer, PCT International Application WO 93/06228 which discloses a variant form of LBP which it terms LBP-β.

One of the normal host effector mechanisms for clearance of bacteria involves the binding to and subsequent phagocytosis by neutrophils and monocytes. As part of this process, bacteria are exposed to bactericidal and bacteriostatic factors, including oxygen radicals, lysosomal enzymes, lactoferrin and various cationic proteins. LBP opsonizes LPS-bearing particles and intact Gram-negative bacteria, mediating attachment of these LBP-coated particles to macrophages. Wright et al., *J. Exp. Med. 170:*1231 (1989). The attachment appears to be through the CD14 receptor of monocytes which binds complexes of LPS and LBP. Wright et al., *Science 249:*1431 (1990). Anti-CD14 mAbs have been shown to block the synthesis of TNF by whole blood incubated with LPS. Wright et al. *Science 249:*1431 (1990). Interaction of CD14, which is present on the surface of polymorphonuclear leukocytes as well as monocytes, with LPS in the presence of LBP has been shown to increase the adhesive activity of neutrophils. Wright et al., *J. Exp. Med. 173:*1281 (1991), Worthen et al., *J. Clin. Invest. 90:*2526 (1992). Thus, while BPI has been shown to be

cytotoxic to bacteria and to inhibit proflammatory cytokine production stimulated by bacteria, LBP promotes bacterial binding to and activation of monocytes through a CD14-dependent mechanism.

LPS, either directly or by inducing proinflammatory cytokines such as IL-1 and TNF, induces the expresion of adhesion molecules including CD54 (intercellular adhesion molecule-1, ICAM-1) and E-selectin (endothelial-leukocyte adhesion molecule-1, ELAM-1) on endothelial cells, and thereby increases binding of leukocytes *in vitro.* Schleimer and Rutledge, *J. Immunol. 136:*649 (1986); Pohlman et al., *J. Immunol. 136:*4548 (1986); Bevilacqua et al., *J. Clin. Invest. 76:*2003 (1985); Gamble et al., *Proc. Natl. Acad. Sci. USA. 82:*8667 (1985); Smith et al., *J. Clin. Invest. 82:*1746 (1988); and Bevilacqua et al., *Proc. Natl. Sci. USA 84:*9238 (1987). However, as CD14 has not been detected on the surface of endothelial cells (Beekhuizen et al., *J. Immunol. 147:*3761 (1990)) and no other receptor for LPS on endothelial cells has been identified, a different mechanism may exist whereby LPS can affect the endothelium.

Soluble CD14, found in serum (Bazil et al., *Eur. J. Immunol. 16:*1583 (1986)), has been hypothesized to be responsible for transmitting the LPS signal to endothelial cells. Specifically, soluble CD14 has been shown to mediate a number of LPS-dependent effects on endothelial cells, including E-selectin and VCAM expression, IL-1, IL-6 and IL-8 secretion, and cell death. Frey et al., *J. Exp. Med. 176:*1665 (1992); Pugin et al., *Proc. Natl. Acad. Sci. USA. 90:*2744 (1993).

Recent studies have shown that soluble CD14 is involved in the LPS-mediated adhesion of neutrophils to endothelial cells. Anti-CD14 mAbs were able to completely inhibit the adhesion induced by LPS,

indicating that the contribution of other CD14-independent LPS receptors to these effects is minimal. The protein(s) on the endothelial cells that soluble CD14 might associate with to transduce the LPS signal remains to be identified. LBP has been shown to be involved in the signal transduction of LPS through soluble CD14; however, at high concentrations of LPS or soluble CD14, LBP does not further enhance the response of endothelial cells to LPS (Pugin et al., *Proc. Natl. Acad. Sci. USA. 90:*2744 (1993).

Larrick et al., *Biochem. and Biophysical Res. Commun.*, *179*:170 (1991) relates to a cationic protein obtained from rabbit granulocytes which is identified as CAP18. CAP18 is identified as bearing no sequence homology with either BPI or LBP. In the course of their disclosure, Larrick et al. characterize other publications which discuss the structure of proteins including LBP and incorrectly attribute to the Wright et al., *supra* disclosure the speculation that "LBP is believed to be composed of two regions: an amino-terminal domain that binds to LPS and a carboxy-terminal domain that may (emphasis supplied) mediate binding of the LBP-LPS complex to the CD14 receptor on leukocytes."

Ulevitch, PCT International Application WO 91/01639 discloses methods and compositions for treatment of sepsis comprising administering anti-CD14 antibodies. The published application also describes "LBP peptide analogs" at page 17 which are stated to be polypeptides capable of competitively inhibiting the binding of LPS-LBP complexes to CD14 expressed on the surface of monocyte derived macrophages. The sequences of the three disclosed "LBP peptide analogs" show 90 to 100% homology with CD14 polypeptide sequences and no homology with LBP sequences.

Ulevitch et al., U.S. Patent No. 5,245,013 discloses a lipopolysaccharide binding protein which binds to Gram-negative bacterially secreted LPS and retards *in vitro* binding of LPS to high density lipoprotein.

Marra, PCT International Application WO 92/03535 discloses various chimeric BPI molecules including an rLBP/BPI chimeric molecule designated LBP25K/BPI30K [LBP(1-197)/BPI(200-456)] and comprising the first 197 amino acid residues of LBP and amino acid residues 200-456 of BPI wherein the coding sequence for the amino-terminal 25 kD portion of LBP was linked to the coding sequence for the carboxy-terminal portion of the BPI protein by virtue of an engineered *ClaI* site within the coding sequence. The resulting molecule reacted positively in an ELISA assay utilizing anti-BPI protein antibodies and also reacted positively in an endotoxin binding assay. Rogy et al., *J. Clin. Immunol.*, *14*: 120-133 (1994) describes experiments utilizing the LBP(1-197)/BPI(200-456) molecule wherein animals treated with the molecule in a primate bacteremia model demonstrated decreased LPS levels compared to controls, but still developed the sequalae of septic shock. For example, no significant reduction in endotoxin mediated cytokine synthesis was observed in endotoxin-treated baboons to whom the compound was administered.

There exists a need in the art for LPS binding and neutralizing proteins which lack CD14-mediated immunostimulatory properties, including the ability to mediate LPS activity through the CD14 receptor.

SUMMARY OF THE INVENTION

The present invention provides novel biologically active polypeptide derivatives of Lipopolysaccharide Binding Protein (LBP), including LBP derivative hybrid proteins, which are characterized by the ability to bind to LPS and which lack CD14-mediated immunostimulatory properties, including the ability of LBP holoprotein to mediate LPS activity via the CD14 receptor. More particularly, LBP protein derivatives including LBP derivative hybrid proteins according to the invention lack those carboxy terminal-associated elements characteristic of the LBP holoprotein which enable LBP to bind to and interact with the CD14 receptor on monocytes and macrophages so as to provide an immunostimulatory signal to monocytes and macrophages.

Presently preferred LBP protein derivatives are characterized by a molecular weight less than or equal to about 25 kD. Particularly preferred LBP protein derivatives of the invention are LBP fragments comprising an amino-terminal region of LBP (e.g., amino acid residues 1-197). A molecule comprising the first 197 amino terminal residues of LBP and designated $rLBP_{25}$ exemplifies the derivatives of the invention. This particular derivative includes amino acid regions comprising LBP residues 17 through 45; 65 through 99 and 141 through 167 which correspond to respective LPS binding domains (e.g., residues 17 through 45, 65 through 99 and 142 through 169) of Bactericidal/Permeability-Increasing protein (BPI).

LBP derivative hybrid proteins of the invention comprise hybrids of LBP protein sequences with the amino acid sequences of other polypeptides and are also characterized by the ability to bind to LPS and the absence of CD14-mediated immunostimulatory properties. Such hybrid

proteins can comprise fusions of LBP amino-terminal fragments with polypeptide sequences of other proteins such as BPI, immunoglobulins and the like. Preferred LBP/BPI hybrids of the invention comprise at least a portion (i.e., at least five consecutive amino acids and preferably ten or more amino acids) of an LPS binding domain of BPI. One preferred LBP derivative hybrid protein of the LBP/BPI type comprises an amino-terminal LBP amino acid sequence selected from within the amino terminal half of LBP (e.g. within amino acid residues 1-197 of LBP) in which one or more portions of that sequence is replaced by the corresponding sequence of BPI selected from within the amino terminal half of BPI (e.g., within amino acid residues 1-199 of BPI). Another preferred LBP derivative hybrid protein comprises a fusion of amino terminal portions of LBP and heavy chain regions of IgG. Other LBP derivative hybrid proteins comprise LBP amino acid sequences into which all or portions of LPS binding domains of e.g., BPI or other LPS binding protein have been inserted or substituted for all or part of an LPS binding region of LBP. Preferred LBP derivative hybrid proteins include those in which all or portions of the previously-noted amino terminal LPS binding domains of BPI replace the corresponding region within LBP.

LBP protein derivatives and LBP derivative hybrid proteins of the invention are expected to display one or more advantageous properties in terms of pharmacokinetics, LPS binding, LPS neutralization and the like.

The present invention further provides novel pharmaceutical compositions comprising the LBP protein derivatives and LBP derivative hybrid proteins along with pharmaceutically acceptable diluents, adjuvants, and carriers and correpsondingly addresses the use of LBP protein

derivatives and LBP derivative hybrid proteins in the manufacture of medicaments for treating gram negative bacterial infections and the sequelae thereof.

Polypeptides of the invention may be synthesized by assembly of amino acids. In addition, the invention provides DNA sequences, plasmid vectors, and transformed cells for producing the LBP protein derivatives and LBP hybrid derivative proteins of the invention.

Numerous additional aspects and advantages of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the invention which describes presently preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the DNA sequence and translated amino acid sequence (1-197) of $rLBP_{25}$ [SEQ ID NOS:1 and 2];

Figure 2 depicts the DNA sequences and translated amino acid sequence (1-456) of rLBP [SEQ ID NOS:3 and 4];

Figure 3 depicts the construction of an $rLBP_{25}$ mammalian expression vector pING4505;

Figure 4 depicts construction of a vector pIC111 encoding an LBP (1-43)/BPI (44-199) hybrid protein;

Figure 5 depicts construction of mammalian expression vectors pING4525 and pING4526 for LBP/BPI hybrid proteins;

Figure 6 depicts construction of plasmids pML105 and pML103 for *in vitro* transcription/translation of LBP/BPI hybrid proteins;

Figure 7 depicts construction of a vector pIC112 encoding a BPI (1-159)/LBP (158-197) hybrid protein;

Figure 8 depicts a graph illustrating the pharmacokinetics of ^{125}I labeled $rLBP_{25}$ and $rBPI_{23}$;

Figure 9 depicts binding of $rLBP_{25}$ to *E. coli* J5 lipid A;

Figure 10 depicts competition by $rBPI_{23}$ and by $rLBP_{25}$ for the binding of ^{125}I-$rLBP_{25}$ to immobilized lipid A;

Figure 11 depicts competition by $rBPI_{23}$ and by recombinant LBP holoprotein (rLBP) for the binding of ^{125}I-rLBP to immobilized lipid A;

Figure 12 depicts the ability of $rLBP_{25}$ and rLBP to inhibit the LAL assay;

Figure 13 depicts the effect of $rLBP_{25}$ and rLBP on binding uptake of ^{125}I-labeled LPS by THP-1 cells;

Figure 14 depicts the effect of $rLBP_{25}$ and rLBP molecules on TNF production by THP-1 cells;

Figures 15A and 15B depict the effect of rLBP on Tissue Factor (TF) and TNF production, respectively, in PBMCs;

Figure 16 depicts the effect of rLBP on TNF production in PBMCs;

Figure 17 depicts the effect of $rLBP_{25}$ on TNF production in PBMCs;

Figure 18 depicts the effect of LBP molecules on Tissue Factor production in PBMCs; and

Figure 19 depicts the effect of $rLBP_{25}$ on LPS induced endothelial adhesiveness for neutrophils;

Figure 20 depicts the effect of rLBP on bacterial binding to monocytes;

Figure 21 depicts the effect of $rLBP_{25}$ on bacterial binding to monocytes;

Figure 22 depicts the binding of rLBP and $rLBP_{25}$ in an LBP sandwich ELISA assay; and

Figure 23 depicts a homology comparison of the amino-terminal amino acid residues of human LBP and human BPI.

DETAILED DESCRIPTION

The present invention encompasses LBP protein derivatives and LBP derivative hybrid proteins which are characterized by the ability to bind to LPS but which lack the carboxy terminal-associated immunostimulatory element(s) characteristic of the LBP holoprotein and thus lack the CD14-mediated immunostimulatory activity characteristic of LBP holoprotein. Preferred LBP protein derivatives are characterized as including N-terminal LBP fragments having a molecular weight of about 25 kD. Most preferred are LBP N-terminal fragments characterized by the amino acid sequence of the first 197 amino acids of the amino-terminus of LBP set out in Figure 1 and SEQ ID NOS:1 and 2. It is also contemplated that LBP protein derivatives containing N-terminal fragments considerably smaller than 25 kD and comprising substantially fewer than the first 197 amino acids of the N-terminus of the LBP holoprotein molecule are suitable for use according to the invention provided they retain the ability to bind to LPS. Thus, specifically contemplated are LBP derivatives comprising part or all of one or more of three regions (defined by LBP amino acid sequences 17-45, 65-99 and 141-167) corresponding (by reason of amino acid homology) to LPS binding regions (comprising amino acid sequences 17-45, 65-99 and 142-169) of BPI. Moreover, it is contemplated that LBP

protein derivatives comprising greater than the first 197 amino acid residues of the holo-LBP molecule, i.e., including amino acids on the carboxy-terminal side of residue 197 of rLBP as disclosed in Figure 2 and SEQ ID NOS:3 and 4 will likewise prove useful according to the methods of the invention provided they lack CD14-mediated immunostimulatory activity. It is further contemplated that those of skill in the art are capable of making additions, deletions and substitutions of the amino-acid residues of SEQ ID NOS:1-4 without loss of the desired biological activities of the molecules. Such, LBP protein derivatives may be obtained by deletion, substitution, addition or mutation, including mutation by site-directed mutagenesis of the DNA sequence encoding the LBP holoprotein, wherein the LBP protein derivative maintains LPS-binding activity and lacks CD14-mediated immunostimulatory activity. One preferred LBP derivative is that wherein the alanine residue at position 131 of the illustrative LBP (1-197) polypeptide fragment is substituted with a cysteine residue. The resulting LBP (1-197) (Cys 131) polypeptide may have the ability to dimerize via interchain disulfide bond formation through cysteine 131 and the resulting dimer may be characterized by improved biological activity.

Also contemplated are LBP derivative hybrid proteins including LBP/BPI hybrid proteins [but excluding the hybrid designated LBP(1-197)/BPI(200-456) noted above] and LBP-Ig fusion proteins which are characterized by the ability to bind LPS but which lack CD-14 immunostimulatory activity. A preferred LBP/BPI hybrid protein of the invention is a protein comprising one or more portion of the amino-terminal half of LBP, e.g., selected from within amino acid residues 1-197 of LBP, and a one or more portions of the amino-terminal half of BPI, e.g., selected from within amino acid residues 1-199 of BPI.

Other LBP hybrid proteins comprise LBP amino acid sequences into which all or portions of LPS binding domains of other LPS binding proteins (such as BPI) have been inserted or substituted. Preferred LBP hybrid proteins include those in which all or portions of the LPS binding domains of BPI (comprising BPI residues 17-45, 65-99 and 142-169) are substituted into the corresponding region of LBP. Portions of such BPI domains substituted into the hybrid proteins may comprise as few as five continuous amino acids but preferably include ten or more continuous amino acids.

LBP derivative hybrid proteins in which all or portions of the LPS binding regions of BPI are substituted into the corresponding region of LBP thus include those comprising at least a part of an LPS binding domain of BPI selected from the group of amino acid sequences consisting of:

ASQQGTAALQKELKRIKPDYSDSFKIKH (SEQ ID NO:17) designated Domain I comprising the amino acid sequence of human BPI from about position 17 to about position 45;

SSQISMVPNVGLKFSISNANIKISGKWKAQKRFLK (SEQ ID NO:18) designated Domain II comprising the amino acid sequence of human BPI from about position 65 to about 99; and

VHVHISKSKVGWLIQLFHKKIESALRNK (SEQ ID NO:19) designated Domain III comprising the amino acid sequence of human BPI from about position 142 to about position 169.

These LPS binding domains of BPI correspond to LBP regions consisting of:

AAQEGLLALQSELLRITLPDFTGDLRIPH (SEQ IS NO:20) comprising the amino acid sequence of human LBP from about position 17 to about position

45;

HSALRPVPGQGLSLSISDSSIRVQGRWKVRKSFFK (SEQ ID NO:21) comprising the amino acid sequence of human LBP from about position 65 to about 99; and

VEVDMSGDLGWLLNLFHNQIESKFQKV (SEQ ID NO 22) comprising the amino acid sequence of human LBP from about position 141 to about position 167.

According to another aspect of the invention, DNA sequences are provided which encode the above-described LBP protein derivatives and LBP derivative hybrid proteins. Also provided are autonomously replicating DNA plasmid vectors including such DNA sequences and host cells stably transformed or transfected with such DNA sequences in a manner allowing their expression. Transformed host cells of the invention are of manifest utility in procedures for the large-scale production of the LBP protein derivatives and LBP derivative hybrid proteins of the invention involving the cultured growth of the hosts in a suitable medium and the isolation of the proteins from the cells or their growth medium.

The invention further provides novel pharmaceutical compositions comprising an LBP protein derivative or an LBP derivative hybrid protein which retains LPS-binding activity and lacks CD14-mediated immunostimulatory activity together with pharmaceutically acceptable diluents, adjuvants, and carriers. The compositions are useful in methods for treating a gram-negative bacterial infection, including the sequelae thereof such as endotoxin related shock, and one or more of conditions associated with gram-negative bacterial infection and resulting endotoxic shock such as disseminated intravascular coagulation, anemia,

thrombocytopenia, leukopenia, adult respiratory distress syndrome, renal failure, hypotension, fever and metabolic acidosis. Such methods comprise administering an LBP protein derivative or LBP derivative hybrid protein to a subject suffering from a gram-negative bacterial infection, including the sequelae thereof.

When employed for treatment of a gram-negative bacterial infection, including the sequelae thereof, LBP protein derivatives and LBP derivative hybrid proteins of the invention are preferably administered parenterally and most preferably intravenously in amounts broadly ranging from about 0.1 milligram and about 100 milligrams per kilogram of body weight of the treated subject with preferred treatments ranging from about 1 milligrams and 25 milligrams per kilogram of body weight. It is contemplated that administration of LBP derivative protein derivatives, such as $rLBP_{25}$, and LBP hybrid proteins may be useful as one aspect of a combination therapy in which BPI or other antibiotics are administered to a subject.

According to a further aspect of the invention, LBP protein derivatives and LBP derivative hybrid proteins may be administered in combination with other therapeutic compositions which are not strictly antibiotics but rather which neutralize the endotoxic effects of LPS such as anti-LPS antibodies and antibodies to constituents of the LPS mediated toxic cascade such as anti-TNF antibodies.

The following detailed description relates to the manufacture and properties of LBP protein derivatives and LBP hybrid proteins of the invention. More specifically, Example 1 relates to the construction of vectors for expression of an exemplary LBP protein derivative, $rLBP_{25}$. Example 2 relates to the construction of vectors for expression of rLBP.

Examples 3 and 4 relate to the incorporation of the vectors of Examples 1 and 2 into appropriate host cells and further describes the expression and purification of $rLBP_{25}$ and rLBP. Examples 5 and 6 relate to construction of vectors encoding LBP/BPI hybrid proteins. Example 7 relates to *in vitro* transcription translation of the LBP/BPI hybrid protein, LBP(1-43)/BPI(44-199) and BPI(1-159)/LBP(158-197). Example 8 relates to the pharmacokinetics of $rLBP_{25}$, rLBP and $rBPI_{23}$ *in vivo*. Example 9 relates to the binding of $rLBP_{25}$ and rLBP to Lipid A. Example 10 relates to competition by $rLBP_{25}$ and $rBPI_{23}$ for the binding of ^{125}I-$rLBP_{25}$ to immobilized lipid A. Example 11 relates to competition by rLBP and $rBPI_{23}$ for the binding of ^{125}I-rLBP to immobilized lipid A. Example 12 relates to the effect of $rLBP_{25}$ and rLBP on an LAL assay. Example 13 relates to the effect of $rLBP_{25}$ and rLBP on the binding/uptake ^{125}I-labeled LPS on TNF production by a human monocyte cell line THP-1. Example 14 relates to the effect of $rLBP_{25}$ and rLBP on TF and TNF production by isolated PBMCs. Example 15 relates to the effect of $rLBP_{25}$ on LPS-induced adhesiveness of endothelial cells for neutrophils. Example 16 relates to the effect of rLBP and $rLBP_{25}$ on bacterial binding to monocytes and polymorphonuclear cells. Example 17 relates to a sandwich ELISA assay for rLBP and $rLBP_{25}$. Example 18 relates to construction of vectors for production of LBP (1-197) (Cys 131). Example 19 relates to construction of vectors for production of LBP-IgG hybrid fusion proteins. Example 20 relates to *in vitro* transcription/translations of truncated LBP fragments and determination of their ability to mediate LPS stimulation of TNF activity. Example 21 relates to construction of vectors for production of LBP/BPI hybrid proteins. Example 22 relates to construction of vectors for production of LBP/BPI hybrid proteins comprising BPI/LBP active

domain replacement and partial replacement mutants. Example 23 relates to properties of synthetic LBP peptides.

EXAMPLE 1

Construction of Vectors for Expression of $rLBP_{25}$ Protein

A. Cloning and sequencing of human $rLBP_{25}$

The DNA encoding amino acids 1-197 of human LBP without the signal sequence (designated "$rLBP_{25}$") was obtained by PCR using human liver poly $(A)^+$ RNA (Clontech Laboratories, Palo Alto, CA) as the source of material for amplification. Reverse transcription of the RNA to cDNA and PCR amplification were carried out using the GeneAmp RNA PCR Kit (Perkin Elmer Cetus, Norwalk, CT) according to the manufacturer's protocols. The sequence of human LBP was obtained from GenBank, accession number M35533, as published by Schumann et al., *Science*, *249*:1429-1431 (1990). The 5' PCR primer corresponded to the amino terminal sequence of the coding region of mature LBP and included a *Bsm*I recognition site at its 5' end. The sequence of this primer, LBP-Bsm, was: 5'-GAATGCAGCCAACCCCGGCT TGGTCGCCA-3' (SEQ ID NO:5). The 3' PCR primer was designed to place a stop codon and an *Xho*I site following the isoleucine at amino acid position No. 197. The sequence of this primer, LBP-2, was: 5'-CTCGAGCTAAATC TCTGTTGTAACTGGC-3' (SEQ ID NO:6). This amino acid was chosen as the endpoint of $rLBP_{25}$ based on sequence homology with an amino-terminal active fragment of BPI (designated $rBPI_{23}$).

The amplified $rLBP_{25}$ DNA was blunt-end cloned into *Sma*I cut pT7T318U (Pharmacia, LKB Biotechnology, Piscataway, NJ) to generate plasmid pIC106. The LBP insert in pIC106 was sequenced using

Sequenase (USB). The $rLBP_{25}$ DNA sequence obtained and the derived amino acid sequence is set out in SEQ ID NOS:1 and 2. The sequence differs in two areas from the sequence of the corresponding region of LBP as published by Schumann et al. (1990) *Science*, *249*:1429-1431, which involve changes in amino acids. These sequence differences are detailed in Table 1.

B. Construction of Vectors for Expression of $rLBP_{25}$ in Mammalian Cells

A vector for the expression of $rLBP_{25}$ using the BPI signal sequence was constructed using the $rLBP_{25}$ coding region sequence isolated from PIC106 by digestion with *Bsm*I, blunt ending with T4 polymerase, and digestion with *Xho*I. This was ligated to two fragments isolated from pING4503 (a plasmid described in co-owned and copending U.S.S.N. 07/885,911 filed May 19, 1992 by Theofan et al. which is hereby incorporated by reference). Briefly, the construction of pING4503 is based on plasmid pING2237N which contains the mouse immunoglobulin heavy chain enhancer element, the LTR enhancer-promoter element from Abelson murine leukemia virus (A-MuLV) DNA, the SV40 19S/16S splice junction at the 5' end of the gene to be expressed, and the human genomic gamma-1 polyadenylation site at the 3' end of the gene to be expressed. Plasmid pING2237N also has a mouse dihydrofolate reductase (DHFR) selectable marker. The two fragments from pING4503 were the vector fragment generated by digestion with *Cla*I and *Xho*I, and a fragment containing the BPI signal sequence generated by digestion with *Eag*I, blunt ending with T4 polymerase, and digestion with *Cla*I. This ligation resulted in a fusion of the BPI signal to the $rLBP_{25}$ coding region, keeping the correct reading frame, and generating pING4505 (DHFR gene). The corresponding *gpt*

vector, designated pING4508, was generated by subcloning the *Sal*I to *Sst*II insert from pING4505 into the *Sal*I and *Sst*II vector fragment from pING3920 (the structure of which is described in co-owned and copending U.S.S.N. 07/718,274 filed June 20, 1991 by Grinna et al., which is hereby incorporated by reference). The construction of pING4505 is outlined in Figure 3.

EXAMPLE 2

Construction of Vectors for Expression of rLBP Proteins

A. Cloning and Sequencing of Human rLBP

The DNA encoding full length human rLBP (amino acids 1-452, designated "rLBP," plus the 25 amino acid signal sequence) was obtained by PCR using human liver poly $(A)^+$ RNA (Clontech Laboratories, Palo Alto, CA) as described for $rLBP_{25}$ above. The 5' PCR primer introduced a *Sal*I recognition site at the 5' end of the LBP signal. The sequence of this primer, LBP-3, was: 5'-CATGTCGACACCATGGGGGCCTTG G-3' (SEQ ID NO:7). The 3' PCR primer was designed to introduce an *Sst*II site following the stop codon of LBP. The sequence of this primer, LBP-4, was: 5'-CATGCCGCGGTCAAACTCTCATGTA-3' (SEQ ID NO:8). The amplified rLBP DNA was blunt-end cloned into *Sma*I cut pT7T318U to generate plasmid pIC128. The LBP insert in pIC128 was sequenced using Sequenase (USB). The rLBP DNA sequence obtained and the derived amino acid sequence are shown in SEQ ID NOS:3 and 4. Additional differences were found between the LBP sequence in pIC128 and the sequence of LBP as published by Schumann et al., *Science 249:*1429-1431 (1990). The rLBP sequence actually encoded 456 amino acids; there was

an insertion of 4 amino acids at position 241. The rLBP sequence in pIC128 additionally contained several nucleotide substitutions that did not change the amino acids compared to Schumann et al. All the differences are highlighted in Table 1. The amino acid sequence is identical to the recently published sequence of LBP_{β} (Seilhamer, PCT International Application WO 93/06228), however, the nucleotide sequence of rLBP herein differs from that of the published LBP_{β} sequence at the sequence encoding amino acids 152 and 179 as shown in Table 1.

Table 1
LBP Sequence Differences

	rLBP Sequence		Schumann LBP Sequence	
Amino Acid Position [1]	Nucleotide	Protein	Nucleotide	Protein
-21	GCC	A	GCA	A
72	CCT	P	CCC	P
129	GTT	V	GGT	G
130	ACT	T	TAC	Y
131	GCC	A	TGC	L
132	TCC	S	CTC	L
148	GAC	D	GAT	D
149	TTG	L	TCG	S
152 [2]	CTG	L	CTC	L
179	TCG/TCA [3]	S	TCA	S
241-245	GTC ATG AGC CTT CCT (SEQ ID NO:9)	VMSLP (SEQ ID NO:10)	GCT	A
411	TTC	F	CTC	L

[1] Amino Acid positions as numbered in SEQ ID NO:4.

[2] This amino acid position not identified as different for LBP-β.

[3] Both nucleotide sequences were found in different PCR-derived clones. This amino acid position also not identified as different for LBP-β.

B. Construction of Vectors for Expression of rLBP in Mammalian Cells

To construct a vector for expression of rLBP in mammalian cells, pIC128 was digested with *Sal*I and *Sst*II, the LBP insert was isolated, and then subcloned into *Sal*I and *Sst*II digested vector pING4222 which is described in co-owned and copending U.S.S.N. 08/013,801 filed February 2, 1993 which is hereby incorporated by reference. The resulting rLBP vector was designated pING4539 and also contained the DHFR gene for selection.

EXAMPLE 3

Production and Purification of rLBP

Mammalian cells are preferred host cells for production of LBP protein derivatives of the invention because such cells allow secretion and proper folding of heterodimeric and multimeric proteins and provide post-translational modifications such as pro-sequence processing and glycosylation.

Mammalian cells which may be useful as hosts for the production of LBP protein derivatives (including LBP/BPI hybrid proteins and rLBP-Ig fusion proteins) include cells of lymphoid origin, such as the hybridoma Sp2/O-Agl4 (ATCC CRL 1581) and cells of fibroblast origin, such as Vero cells (ATCC CRL 81), CHO-K1, CHO-DXB11, or CHO-DG44. The latter cell line (a DHFR$^-$ mutant of CHO Toronto obtained from Dr. Lawrence Chasin, Columbia University) was maintained in Ham's F12 medium plus 10% fetal bovine serum supplemented with glutamine/penicillin/streptomycin (Irvine Scientific, Irvine, CA).

CHO-DG44 cells were transfected with linearized pING4539 DNA (40 μg, digested with *Pvu*I, phenol-chloroform extracted and ethanol precipitated) using electroporation. Following recovery, the cells were diluted and 1 x 10^4 cells were plated per 96-well plate well in selective medium consisting of an αMEM medium lacking nucleosides (Irvine Scientific) and supplemented with dialyzed fetal bovine serum (100 ml serum dialyzed using 4L cold 0.15 NaCl using 6000-8000 cutoff for 16 hours at 4°C). Untransfected CHO-DG44 cells are unable to grow in this medium because they possess the DHFR$^-$ mutation and were removed during successive feedings with the selective medium. At 1.5-2 weeks, microcolonies consisting of transfected cells were observed.

Clones were analyzed for the presence of LBP-reactive protein in culture by ELISA using Immulon-II 96 well plates (Dynatech). Supernatant samples were added to the plates, incubated 46 hours, 4°C followed by goat anti-LBP antiserum and peroxidose-labeled rabbit anti-goat anti-serum. The 21 most productive positive clones were expanded in selective αMEM medium and then grown on selective medium supplemented with 0.05μM methotrexate. The best producing amplified clone was chosen based on ELISA of supernatants as described above and then expanded in αMEM media containing 0.05μM methotrexate for growth in roller bottles.

rLBP was produced in CHO-DG44 cells transfected with the plasmid pING-4539. All incubations were performed in a humidified 5% CO_2 incubator maintained at 37°C. Working stock cultures were grown in DME/F-12 with 10% FCS, and were then seeded into 40 2-liter roller bottles (500 mL per bottle) at a density of 9.6 x 10^4 cells/mL in DME/F-12 with 5% FCS (4.8 x 10^7 cells/bottle). Four days later, the culture

supernatants from each bottle were removed and then replaced with 500 mL of fresh media (DME/F-12 with 2.5% FCS). Ten mL of an S-Sepharose (Pharmacia) ion exchange resin slurry (50% v/v, sterilized by autoclaving) was then added to each bottle, and incubation was continued. After 2 days, the media containing S-Sepharose was harvested and replaced with fresh media again containing S-Sepharose. This process was repeated one more time, to yield three harvests of S-Sepharose over a four day period. After each harvest, the S-Sepharose resin was allowed to settle for 1 hour, and the spent media was removed by aspiration. The sedimented S-Sepharose was resuspended in fresh media and pooled. The method of purification using S-Sepharose has been described and claimed in co-owned and copending U.S. Patent Application Serial No. 07/855,501 and in co-owned and copending PCT US93/04752 filed May 19, 1993, the disclosures of which are hereby incorporated by reference in their entirety.

All chromatographic resins used in the purification of rLBP and $rLBP_{25}$ were depyrogenated by immersion in 0.2N NaOH, 1 M NaCl and then rinsed with pyrogen-free water All buffers and reagents were prepared with bottled, pyrogen-free water for irrigation (Baxter).

The S-Sepharose resin harvested from culture supernatants was washed successively with 5 volumes of cold 20 mM MES, 0.1 M NaCl, pH 6.8 (two times), 20 mM NaAc, 0.1 M NaCl, pH 4.0 (two times), and 20 mM NaAc, 0.4 M NaCl, pH 4.0 (two times). For each of these sedimentations, the resin (in 1 L roller bottles) was allowed to settle at 1 x g in the cold. The resin from each harvest (100-200 mL settled volume) was next packed into individual 5 x 10 cm columns and equilibrated in 20 mM NaAc, 0.4 M NaCl, pH 4.0 at 4°C. Two of the columns were subsequently washed with 20 mM NaAc, 0.7 M NaCl, pH

4.0, until the absorbance at 280 nm approached zero, and were then batch eluted with 20 mM NaAc, 1.0 M NaCl, pH 4.0. Eluted rLBP was identified by SDS-PAGE in 12.5% gels. These gels also showed that some of the rLBP has eluted in the 0.7 M NaCl wash. Consequently, the third batch of resin (about 300 mL) was eluted with a gradient of NaCl (0.4 M to 1.0 M) in 20 mM NaAc, pH 4.0, and 8.5 mL fractions were collected. SDS-PAGE analysis of column fractions indicated that rLBP eluted between fractions 70 and 100. It was estimated that the amount of total protein present in all three eluates was approximately 800 mg.

The S-Sepharose fractions containing rLBP from the three harvests were pooled (850 mL), and one-half of the material (425 ml, or about 400 mg rLBP) was purified further. To this material was added 1275 mL of 20 mM NaAc, pH 4.0, such that the final concentration of NaCl would be approximately 0.25 M, and the solution was applied to a second S-Sepharose column (2.5 x 30 cm) equilibrated at 4°C in 20 mM NaAc, 0.4 M NaCl, pH 4.0. The column was eluted with a gradient of NaCl (0.4 to 1.0 M) in 20 mM NaAc, pH 4.0, and column fractions containing rLBP were again identified by SDS-PAGE and pooled. The final volume of pooled material was 133 ml, and it contained about 260 mg of rLBP.

Final purification of rLBP was accomplished by chromatographing the eluate from the second S-Sepharose column on a 345 mL Sephacryl S-100 size exclusion column equilibrated in 20 mM NaCitrate, 0.15 M NaCl, pH 5.0 at 4°C. Five 25 mL aliquots of the S-Sepharose eluate were successively applied to, and eluted from, the column, and 3.7 mL fractions were collected. SDS-PAGE analysis of the effluent fractions indicated that rLBP eluted as a single band, and peak

fractions were pooled and stored at 4°C. The peak fractions from all five S-100 runs were then pooled. The final material (about 245 mg) was >99% pure by SDS-PAGE, and exhibited a molecular weight of about 60 kD.

EXAMPLE 4

Production and Purification of $rLBP_{25}$

CHO-DG44 cells were transfected with linearized pING4505 DNA (40 μg, digested with *Pvu*I, phenol-chloroform extracted and ethanol precipitated) using the calcium phosphate method of Wigler, *et al.*, *Cell*, *11*:223 (1977). Following calcium phosphate treatment, the cells were plated in T75 flasks and transfectants were obtained by growth in selective medium consisting of an αMEM medium lacking nucleosides (Irvine Scientific) and supplemented with dialyzed fetal bovine serum (100 ml serum dialyzed using 4L cold 0.15 NaCl using 6000-8000 cutoff for 16 hours at 4°C). Untransfected CHO-DG44 cells are unable to grow in this medium because they possess the $DHFR^-$ mutation and were removed during successive feedings with the selective medium. At 1.5-2 weeks, only microcolonies consisting of transfected cells were observed. The transfected cells were removed from the flasks by trypsinization and subcloned by limiting dilution in 96 well plates.

Subclones were analyzed for the presence of $rLBP_{25}$ protein in culture supernatants by anti-gamma ELISA using Immulon-II 96 well plates (Dynatech) with LPS as a pre-coat, followed by culture supernatant, goat anti-LBP anti-serum and peroxidase-labeled rabbit anti-goat antiserum. The positive clones were also retested for LBP specific mRNA. The top

producing clone was expanded in selective αMEM medium for growth in roller bottles.

$rLBP_{25}$ was produced in CHO-DG44 cells transfected with the plasmid pING4505 following procedures similar to those described above in Example 3 for rLBP, with minor modifications. After the harvested S-Sepharose was washed in 20 mM NaAc, 0.4 M NaCl, pH 4.0, $rLBP_{25}$ was batch eluted by the addition of 20 mM NaAc, 1.0 M NaCl, pH 4.0. To the pooled 1.0 M NaCl eluates from the 3 harvests (862 mL) was added 137 mL of 0.5 M MES, such that the final volume was 999 mL, and the final NaCl concentration was about 0.87 M. At this stage, it was estimated that the amount of total protein present was about 775 mg.

A portion of S-Sepharose eluate (549 mL) was diluted with water such that the final volume was 1600 mL, and the final NaCl concentration was about 0.3 M. This diluted material was applied to a 2.5 x 20 cm column of Q-Sepharose previously equilibrated in 20 mM MES, 0.2 M NaCl, pH 5.5 at 4°C. The eluate flowing through the column (which contains the $rLBP_{25}$) was then applied to a second S-Sepharose column (2.5 x 30 cm), and the column was eluted with a gradient of 0.4 M to 1.2 M NaCl in 20 mM NaAc, pH 4.0. $rLBP_{25}$-containing fractions were identified by SDS-PAGE and peak fractions were pooled. This pooled material (60 mL) was divided into 20 mL aliquots and sequentially chromatographed on Sephacryl S-100. Peak fractions from the three runs were pooled and rechromatographed over the same S-100 column. The final material (about 33 mg) was >99% pure by SDS-PAGE, and exhibited a molecular weight of about 22 kDa.

EXAMPLE 5

Construction of Vectors for Expression of LBP (1-43)/BPI (44-199) Hybrid Protein

In this example vectors encoding a hybrid protein [LBP (1-43)/BPI (44-199) hybrid] comprising the first 43 amino-terminal amino acids of LBP and amino acid residues 44-199 of BPI (for BPI see [SEQ ID NOS:11 AND 12] were constructed.

A. Construction of Intermediate Vector pIC111

Intermediate vector pIC111 was constructed according to the following procedure as set out in Figure 4. A 250 bp fragment was isolated from pING4505 that encoded the BPI signal sequence and amino acids 1-43 of LBP by digesting with BamHI, blunting the end with T4 polymerase, then cutting with *Sal* I. To generate a fragment of BPI that would provide a blunt junction beginning with amino acid 44, a PCR fragment was produced using two primers. The sequence of primer BPI-18 was 5' AAGCATCTTGGGAAGGGG 3' [SEQ ID NO:13] and the sequence of primer BPI-11 was 5' TATTTTGGTCATTACTGGCAGAGT 3' [[SEQ ID NO:14]. The BPI-containing plasmid pING4502 was used as template. Plasmid pING4502 is essentially the same as plasmid pING4503, but does not contain the 30 bp of the 5' UT region of BPI and has the gpt gene instead of DHFR gene for selection. The PCR amplified DNA was digested with *Afl*II and the resulting 100 bp fragment, encoding BPI residues 44 through about 76 was purified. This fragment was ligated together with the 250 bp BPI signal/LBP 1-43 fragment described above into the *Sal*I - *Afl*II vector fragment from pIC110 (BPI in T7T3) to generate pIC111.

B. Construction of Mammalian Expression Vector pING4525

The insert in pIC111 encoding LBP (1-43)/BPI (44-199) with BPI signal was digested with *Sal*I and *Sst*II and subcloned into *Sal*I - *Sst*II digested pING4502 vector to generate mammalian expression vector pING4525. The construction of pING4525 is set out in Figure 5.

C. Construction of Vector for *In Vitro* Transcription, Translation (pML105)

The construction of *in vitro* transcription plasmid pML105 is set out in Figure 6. pING4525 was digested with *Not*I, treated with mung bean nuclease to blunt the end, then digested with *Xho*I. The resulting 600 bp fragment, which encodes LBP (1-43)/BPI (44-199) without a signal sequence, was gel purified. This fragment was subcloned into pIC127 plasmid which had been digested with *Nco*I, treated with T4 polymerase then *Xho*I, to generate pML105. pIC127 is essentially pGEM1 (Promega, WI) with a BPI_{23} insert cloned downstream of the SP6 RNA polymerase promoter designed for *in vitro* transcription of the BPI. Digestion of pIC127 with *Nco*I followed by T4 polymerase (to fill in) generates an ATG that can be joined in a blunt junction to a sequence that can then be transcribed in a cell free system.

EXAMPLE 6

Construction of Vectors for Expression of BPI (1-159)/LBP (158-197) Hybrid Protein

In this example a vector encoding a hybrid protein [BPI (1-159)/LBP (158-197)] comprising the first 159 amino terminal amino acids of BPI and amino acid residues 158-197 of LBP was constructed.

A. Construction of intermediate pIC112.

Plasmid pING4505 was digested with *Ear*I and *Sst*II and the 135 bp fragment corresponding to LBP 158-197 (plus stop condon) was gel purified. This fragment was ligated with 2 fragments from pIC110, the 250 bp *Afl*II - *Ear*I fragment and the vector fragment (*Sst*II - *Afl*II) to generate pIC112 as set out in Figure 7.

B. Construction of Mammalian Expression Vector pING4526.

The insert encoding BPI (1-159)/LBP (158-197) was excised from pIC112 with *Sal*I and *Sst*II and subcloned into *Sal*I - *Sst*II digested pING4502 to generate pING4526 as set out in Figure 5.

C. Construction of Vector for *In Vitro* Transcription/Translation (pML103)

pING4526 was used as the source of a 430 bp *Eco*RI- *Sst*II fragment (encoding BPI 61-159/LBP 158-197). This fragment was subcloned into the *Sst*II - *Eco*RI vector fragment from pIC127 to generate pML103, encoding the BPI (1-159)/LBP (158-197) insert in a vector for *in vitro* transcription. This construction is set out in Figure 6.

EXAMPLE 7

In Vitro Transcription/Translation and Lipid A Binding Activity of LBP/BPI Hybrid Protein

In this example, *in vitro* transcription/translation reactions were carried out for plasmids encoding BPI_{23} and the LBP/BPI hybrid proteins of Examples 5 and 6. Specifically, the TNT™ Coupled Reticulocyte Lysate System Kit (Promega, WI) with SP6 RNA polymerase was used to carry out *in vitro* transcription/translation of plasmids pML103 and pML105 to produce hybrid LBP/BPI proteins. The kit is a complete

system, which provides all the reagents necessary to make protein. One adds only the DNA template specifying the protein sequence of interest, a radiolabeled amino acid (^{35}S-methionine) and a ribonuclease inhibitor (Promega's recombinant RNasin).

The protocol used to generate the LBP/BPI hybrid proteins was essentially that suggested by Promega's Technical Bulletin #126. Reactions were prepared in volume of 50 μL containing: TNT Rabbit Reticulocyte Lysate, TNT Reaction Buffer, TNT SP6 RNA Polymerase, amino acid mixture minus Methionine, ^{35}-S Methionine used was *in vivo* cell labelling grade purchased from Amersham (catalog #SJ.1015). One microgram of DNA from either pML103 or pML105 was put into the *in vitro* transcription-translation system. As specified by Promega, the transcription-translation reactions were incubated for 2 hours in a water bath at 30°C, then analyzed in various ways to assess the protein products made.

The amounts and molecular weights of the LBP/BPI hybrid proteins were determined by SDS PAGE/Phosphorimager analysis (Molecular Dynamics, CA), TCA precipitation and ELISA. The binding of the LBP/BPI hybrid proteins to immobilized J5 Lipid A was also measured.

A BPI sandwich ELISA was carried out using rabbit anti-BPI as the capture antibody on the plate and biotin labeled anti-BPI followed by alkaline phosphatase conjugated to streptavidin as the detection system.

A J5 Lipid A binding (RIA) binding assay was carried out using pop-apart Immulon-2 strips coated overnight with 25 ng per well of J5 Lipid A. Two sets of plates (coated and uncoated) were blocked for three hours at 37°C with 0.1% BSA/PBS, washed with PBS/0.05% Tween, then binding was done at 37°C for one hour with 50 μL of a 1:25 dilution

of the transcription-translation reactions. After binding, plates were washed, then placed in scintillation vials with 2 mL of scintillation fluid and counted in a (Beckman) beta-counter.

Comparable amounts of LBP/BPI hybrid protein of the anticipated molecular weight were made for each of the analogs. However, when the total protein in each reaction was quantitated by ELISA, the yields appeared to differ. The absolute numbers obtained in the ELISA may reflect altered epitopes in the LBP/BPI hybrid proteins which may not be recognized equally by the rabbit anti-BPI antibody. In particular, the low amount of pML105 detected by ELISA is thought to underestimate the actual protein yield. The LBP/BPI hybrid proteins bound immobilized J5 Lipid A as well as BPI_{23} (1-199) made with pIC127. Specific binding (background corrected) for the LBP/BPI hybrid proteins is shown in Table 2. LBP/BPI hybrid proteins of the invention may have such desired properties as LPS-binding activity comparable to $rBPI_{23}$, while lacking CD14-mediated immunostimulatory activity characteristic of the holo-LBP proteins, and may have advantageous pharmacokinetic properties such as increased half-life as compared with $rBPI_{23}$ as measured in Example 8 below.

TABLE 2

Plasmid	Description	Yield ELISA	Specific Binding RIA, J5 Lipid A
pIC127	BPI23 (1-199)	57 ng	140,000 cpms
pML103	BPI (1-159) LBP (158-197)	100 ng	210,000 cpms
pML105	LBP (1-43) BPI (44-199)	12 ng	150,000 cpms

EXAMPLE 8

Pharmacokinetics of $rLBP_{25}$, rLBP and $rBPI_{23}$ in Male CD Rats

(The pharmacokinetics of $rLBP_{25}$, rLBP and $rBPI_{23}$ were investigated in rats using an ELISA assay.) The pharmacokinetics of 1 mg/kg $rLBP_{25}$ was investigated in 3 male CD rats. Blood samples were collected at selected times (from 0.5 minutes to 72 hours) after administration of dose. Plasma samples were then assayed by ELISA using affinity purified rabbit anti-LBP_{25} as the capture antibody and biotin labelled affinity purified rabbit anti-LBP_{25} as the secondary antibody.

The plasma concentration-time profile of $rLBP_{25}$ could be fit to a three-compartment model, with α half-life of 2.3 $\pm$ 0.3 minutes, β half-life of 10.8 $\pm$ 1.0 minutes, and γ half-life of 101 $\pm$ 14 minutes, with a systemic mean residence time of about 9.2 $\pm$ 0.8 minutes (Table 3). The rate of clearance of $rLBP_{25}$ from the plasma was about 10 times faster than

that observed for rLBP, and about 5 times slower than the clearance rate of $rBPI_{23}$. Unlike BPI, removing the carboxy terminal end of LBP significantly increased its clearance rate. However, the steady state volume of distribution of LBP_{25} is similar to that of LBP, suggesting that the distribution of LBP was not affected by this modification.

The pharmacokinetics of 1 mg/kg rLBP was investigated in 3 male CD rats. Blood samples were collected at selected times (from 0.5 minutes to 75 hours) after administration of dose. Plasma samples were then assayed by ELISA using affinity purified rabbit anti-LBP_{25} as the capture antibody and biotin labelled affinity purified rabbit anti-LBP_{25} as the secondary antibody.

The plasma concentration-time profile of rLBP could be fit to a three-compartment model, with α half-life of 14 $\pm$ 2 minutes, β half-life of 130 $\pm$ 16 minutes, γ half-life of 561 $\pm$ 37 minutes (Table 3). The rate of clearance of rLBP from the plasma was very slow, about 60 times slower than $rBPI_{23}$.

Table 3

Pharmacokinetics Parameter Values from the Serum Clearance Curve of 1 mg/kg $rBPI_{23}$, $rLBP_{25}$ and rLBP
(mean ± se)

Test Article	Vc mL/kg	Vss mL/kg	Clearance mL/min/kg	MRT minutes	$t_{1/2}\ \alpha$ minutes	$t_{1/2}\ \beta$ minutes
$rBPI_{23}$	62.3 ± 15	78 ± 16	25.5 ± 5.4	3.0 ± 0.0	1.62 ± 0.06	18.0 ± 3.0
$rLBP_{25}$	46.8 ± 0.6	269 ± 60	5.1 ± 0.4	51.8 ± 8.9	2.33 ± 0.3	10.8 ± 1.0
rLBP	55.7± 9.0	208 ± 77	0.45 ± 0.1	437.6 ± 55	13.57 ± 2.0	129.7 ± 16

Vc = volume of distribution of the central compartment.

Vss = steady state volume of distribution.

MRT = total body mean residence time.

$t_{1/2}\ \alpha$ = alpha half-life.

$t_{1/2}\ \beta$ = beta half-life.

EXAMPLE 9

Binding of $rLBP_{25}$ and rLBP to Lipid A

In this example, the binding of $rLBP_{25}$ to immobilized lipid A was determined according to the method of Gazzano-Santoro et al., *Infect. Immun. 60:* 4754-4761 (1992) but utilizing ^{125}I labeled $rLBP_{25}$ instead of ^{125}I labeled $rBPI_{23}$. Radioiodination of proteins was performed essentially as previously described (Gazzano-Santoro et al.) except that the iodination was performed in the absence of Tween 20 and the iodinated protein was exchanged by gel filtration into 20 mM citrate, pH 5.0, 0.15M NaCl, 0.1% F68 (Poloxamer I88) 0:002% Polysorbate 80.

Specifically, an *E. coli* J5 lipid A suspension was sonicated, diluted in methanol to a concentration of 0.1 μg/mL and 100 μL aliquots were allowed to evaporate in wells (Immulon 2 Removawell Strips, Dynatech) at 37°C overnight. The wells were then blocked with 215 μL of D-PBS/0.1% BSA (D-PBS/BSA) for three hours at 37°C. The blocking buffer was discarded, the wells were washed in D-PBS/0.05% Tween 20 (D-PBS/T) and incubated overnight at 4°C with D-PBS/T containing increasing amounts of ^{125}I labeled $rLBP_{25}$, or $rBPI_{23}$ as described in Gazzano-Santoro et al. After three washes, bound radioactivity was counted in a gamma counter. The binding to wells treated with (D-PBS/BSA) only was taken to represent nonspecific binding; specific binding was defined as the difference between total and nonspecific binding. For rLBP, the experiment was performed as described above except that the wells were blocked in D-PBS/1%BSA, washed with D-PBS/0.1%BSA and the binding incubation buffer was D-PBS/0.1% BSA. The resulting data were fitted to a standard binding equation by computerized non-linear curve

fitting (Leatherbarrow, *Trends Biochemical Sciences*, *15*: 455-458 (1990); GraFit Version 2.0, Erathicus Software Ltd., Staines, UK). According to these experiments the labeled $rLBP_{25}$ had a K_d of about 76 nM (Figure 9) and the rLBP had a K_d of about 60 nM, while $rBPI_{23}$ had a K_d of approximately 3 nM, indicating an approximately 15-25 fold lower binding affinity of rLBP25 and rLBP, as compared with $rBPI_{23}$, for LPS. However, the binding of $rLBP_{25}$ and rLBP were comparable, demonstrating that the LBS-binding site of rLBP is localized in the N-terminal portion of the molecule.

EXAMPLE 10

Competition by $rBPI_{23}$ and by $rLBP_{25}$ for the Binding of ^{125}I-$rLBP_{25}$ to Immobilized Lipid A

In this example, the inhibition of ^{125}I-labeled $rLBP_{25}$ binding to *E. coli* J5 lipid A by unlabeled $rBPI_{23}$ or $rLBP_{25}$ was determined. Specifically, *E. coli* J5 lipid A was diluted in methanol to a concentration of 1 μg/mL and 50 μL aliquots were allowed to evaporate in wells overnight at 37°C. The wells were then blocked in D-PBS/BSA. Increasing amounts of unlabeled $rBPI_{23}$ or $rLBP_{25}$ in a 50 μL volume were then added and the wells were incubated overnight at 4°C in D-PBS/T. Twenty microliters of ^{125}I-$rLBP_{25}$ (0.65×10^6 cpm, specific activity 5.29 μCi/μg) were added directly to each well and further incubated for one hour at 4°C. After three washes in D-PBS/T, wells were counted. Figure 10 shows that although $rLBP_{25}$ is able to compete with ^{125}I-$rLBP_{25}$ for binding to lipid A, $rBPI_{23}$ is a better competitor. These results are consistent with the difference in relative affinity as shown in Example 9.

EXAMPLE 11

Competition by $rBPI_{23}$ and by rLBP for the Binding of ^{125}I-rLBP to Immobilized Lipid A

In this example, the inhibition of ^{125}I-labeled rLBP binding to *E. coli* J5 lipid A by $rBPI_{23}$ and rLBP was determined. Specifically, *E. coli* J5 lipid A was diluted in methanol to a concentration of 1 μg/mL and 100 μL aliquots were allowed to evaporate in wells overnight at 37°C. Following incubation, the wells were blocked in D-PBS/1%BSA. Increasing amounts of unlabeled $rBPI_{23}$ or rLBP mixed with a fixed amount of ^{125}I-rLBP (1.45×10^6 cpm, specific activity 1.74 μCi/μg) were added to wells in a 100μL volume and incubated overnight at 4°C in D-PBS/BSA. After three washes in D-PBS/BSA, wells were counted. Figure 11 shows the results wherein increasing amounts of both rLBP and $rBPI_{23}$ compete with radiolabeled rLBP binding for the immobilized lipid A. These results show that $rBPI_{23}$ was more potent than rLBP at competing for rLBP (Figure 11) binding to lipid A consistent with the difference in relative affinity as shown in Example 9.

EXAMPLE 12

Ability of $rLBP_{25}$ and rLBP to Inhibit the LAL Assay

In this example, the effect of $rLBP_{25}$ and rLBP were compared for their ability to inhibit the *Limulus* amebocyte lysate (LAL) assay. Specifically, increasing concentrations of the LPS binding proteins were incubated in the presence of 2 ng/mL of *E. coli* 0113 LPS (60 μL volume) for three hours at 37°C. The samples were then diluted with PBS to bring the LPS concentration to 333 pg/mL and the amount of LPS activity was determined in the chromogenic LAL assay (Whittaker

Bioproducts, Inc.). The results shown in Figure 12 show that $rLBP_{25}$ and the rLBP protein products had comparable endotoxin neutralizing activity in the LAL assay.

EXAMPLE 13

Effect of LBP Molecules on Binding/Uptake of ^{125}I-labeled LPS and on TNF Production by THP-1 Cells

In this example, the effect of $rLBP_{25}$ and rLBP on binding/uptake of LPS and TNF production by a human monocyte cell line bearing CD14 receptors on its cell surface was determined. Specifically, THP-1 human monocyte cells (obtained from the American Type Culture Collection Tumor Immunology Bank, 12301 Parklawn Dr., Rockville, MD 20852) were grown to a density of 3.5×10^5/mL in RPMI 1640 media (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (Hyclone Laboratories, Logan, UT), 1mM glutamine, 1 mM pyruvate (Gibco), and 10U/mL penicillin and streptomycin (Gibco). The cells were transferred at the same density into the above medium supplemented with 100nM 1,25 dihydroxyvitamin D_3 (Biomol Research Laboratories, Plymouth Meeting, PA) and allowed to grow for another 3 days before use. Tubes containing 2×10^6 cells in 1 mL of RPMI/0.1% BSA were incubated in the presence of 20 ng/mL of ^{125}I-labeled Ra LPS (List Biological Laboratories, Campbell CA) with various concentrations of $rLBP_{25}$, or rLBP. Nonspecific uptake was determined by adding a 2000-fold excess of unlabeled RaLPS for one hour at 37°C. At the end of the incubation time, the cells were removed from the binding medium by centrifugation through a layer of dibutyl/dioctyl

phthalate oil and counted. Supernatants were sampled at the end of the assay to determine TNF production.

The presence of rLBP stimulated both the uptake of ^{125}I-labeled LPS (Figure 13) and the release of TNF (Figure 14) by the THP-1 cells. In contrast, if the cells are incubated with $rLBP_{25}$, there was no significant uptake of ^{125}I-labeled LPS (Figure 13) and no significant TNF production (Figure 14).

EXAMPLE 14

Effect of LBP Molecules on TF and TNF Production in PBMCs

In this example, peripheral blood mononuclear cells (PBMCs) isolated from healthy human donors were used in a model system to determine the effects of $rLBP_{25}$ and rLBP on LPS-mediated effects. PBMCs include approximately 20% monocytes which are known to produce tissue factor (TF) which is a cell surface protein responsible for initiating blood clotting and tumor necrosis factor (TNF) when stimulated by LPS Tissue Factor if assembled with factor VII initiates the blood coagulation cascades. TF activity induced by LPS has been implicated in the pathogenesis of disseminated intravascular coagulation (DIC), which is a major complication of gram-negative sepsis. Bone, *Annals Int. Med. 115:*457 (1991).

According to the experimental protocol, PBMCs were prepared from buffy coat from the blood of healthy human donors by separation over Ficoll-Hypaque. The percentage of monocytes (15-25%) in different PBMC preparations was determined by fluorescence activated cell sorting (FACS) analysis based on the expression of CD14 antigen. The

PBMCs (2 x 10^6/mL) were incubated in 1 mL Dulbecco's modified Eagle medium containing 25 mM HEPES, at 37°C, 5% CO_2 for 4 hours. During this incubation, LPS in varying concentrations is present with varying concentrations of rLBP or $rLBP_{25}$ or 20% fetal bovine serum (FBS). After incubation the cells and media were separated by centrifugation. The cells were washed with Tris-NaCl buffer (0.1 M Tris, 0.15M NaCl, 0.1% bovine serum albumin, pH7.4) then lysed in the same buffer by adding 15mM octyl-beta-D-glycopyranoside and incubated at 37°C for 30 minutes.

To determine TNF production by the PBMCs incubated as described above, the supernatant of the PBMCs was assayed for TNF by ELISA (T Cell Sciences, Cambridge, MA).

To determine TF production, total cellular TF activity was determined in the PBMC lysate using a two-stage amidolytic assay, similar to that described by Moore et al., *J. Clin. Invest. 79:*124-130 (1987). In the first stage, the PBMC lysate was mixed with coagulation factor VII and factor X, in order to combine TF with factor VII, which in turn, activated factor X. In the second stage the activity of factor Xa was measured using a chromogenic peptide substrate. Factor VII, factor X and this synthetic peptide substrate (Spectrozyme FXa [MeO-CO-D-CHG-Gly-Arg-pNA)] were the products of American Diagnostica, Greenwich, CT. TF activity was calculated by reference to standard curves obtained with dilutions of rabbit brain thromboplastin (Sigma Chemical Co., St. Louis, MO) in the amidolytic assay.

For this assay in a 96 well microtiter plate, cell lysate (containing 10^3-10^4 monocytes) or different dilutions of the thromboplastin standard (corresponding to 0.1 to 1 μl buffer (0.1 M Tris, 0.15 M NaCl,

pH 7.4) containing 7 mM $CaCl_2$. The mixture was incubated at room temperature for 20 min. Then 50 μl of Spectrozyme FXa, (synthetic substrate for factor Xa; 1.4 mg/ml) was added and the rate of absorbance increase at 405 nm was measured using a kinetic plate reader (Molecular Devices, Menlo Park, CA).

According to one experiment, varying amounts of recombinant rLBP (produced according to the method of Example 3) and 100 or 1000 pg/ml of *E. coli* LPS were contacted with the isolated PBMCs to determine the effect of rLBP on the appearance of TF in the cell lysate and on TNF release. The results in Figures 15a and 15b show that rLBP greatly stimulates the LPS-mediated synthesis of TF and TNF. Similarly, incubation of LPS (100 or 1000 pg/ml) and 20% fetal bovine serum (FBS) also resulted in TF and TNF synthesis. Since LBP is present in serum, the bar-graphs at the right of Figures 15a and 15b show that serum also mediates LPS mediated responses of TF and TNF production. To determine whether the effects of LPS are mediated by the CD14 antigen present on the surface of monocytes, antibodies to this determinant were included in the incubation mixture in other experiments. Addition of anti-CD14 monoclonal antibody (MY4; Coulter Immunology, Hialeah, FL) in the presence of serum or rLBP inhibited the induction of both tissue factor activity and TNF release. The extent of inhibition diminished at higher LPS concentrations.

The use of the PBMC model to determine TF and TNF production was then repeated to compare the effect of $rLBP_{25}$ with that of recombinant rLBP on LPS-induced TF and TNF production. The results with varying concentrations of LPS shown in Figure 16 compare administration of 20% FBS with serum-free media containing various

concentrations of recombinant rLBP. These results again demonstrate that rLBP potentiates TNF release. The results shown in Figure 17 with varying concentrations of LPS compare administration of 20% FBS with varying concentrations of $rLBP_{25}$ in serum-free media. In contrast to the results with rLBP, these results demonstrate that $rLBP_{25}$ does not have an immunostimulatory effect and therefore does not potentiate the release of TNF.

Although $rLBP_{25}$ could not mediate the CD14-dependent LPS stimulation of monocytes to produce TF or TNF, the ability of $rLBP_{25}$ to compete with rLBP and inhibit the LPS-mediated appearance of TF on the PBMC cell surface was tested. Specifically, $rLBP_{25}$ produced according to the method of Example 4 was added in concentrations ranging from 0.1 to 10.0 μg/mL to PMBCs that were incubated with a constant amount of rLBP (2 μg/ml) and stimulated with either 20 pg/mL or 200 pg/mL LPS. The results shown in Figure 18 demonstrate that not only does $rLBP_{25}$ not mediate LPS-stimulated TF production similar to rLBP but also it effectively competes with rLBP to substantially reduce the LPS-induced TF production. Similar effects were obtained for TNF production.

EXAMPLE 15

Effect of $rLBP_{25}$ on LPS Induction of Endothelial Cell Adhesiveness for Neutrophils

In this example, the effect of $rLBP_{25}$ on LPS induction of endothelial cell adhesiveness for neutrophils was studied in an *in vitro* adherence assay. Human umbilical cord endothelial cells (HUVEC) were cultured in a 48-well plate and incubated for 4 hours at 37°C with 10

ng/mL LPS (*E. coli* 0113) in M199 supplemented with 2% FCS, or with the medium only. $rLBP_{25}$ was added to the LPS-containing wells at concentrations varying from 0.1 to about 100μg/mL to determine its ability to neutralize the LPS-induced increase in the adhesiveness of endothelial cells. Following the four hour incubation, HUVEC monolayers were washed three times, and 2.5 x 10^5 ^{51}Cr-labeled human blood neutrophils in 0.2mL RMPI-2% FCS were added to each well. The plate was incubated at 37°C for 30 minutes. After the incubation, the supernatant was aspirated and each well was washed with 1 mL of warm medium to remove non-adherent neutrophils. The cells remaining in each well were solubilized with 0.2 mL of 0.25N NaOH and the lysates were counted in a gamma counter. Percent neutrophil adherence was calculated as 100 x cpm lysate/cpm added. The data presented in Figure 19 on the mean of percent neutrophil adherence of four replicates per group. The results shown in Figure 19 demonstrate that $rLBP_{25}$ at 100 μg/ml completely inhibits the LPS-induced neutrophil adhesion. Under the conditions of this assay, 10 or 1 μg/mL of $rLBP_{25}$ inhibits 71% and 51%, respectively, of the LPS-stimulated adherence, while 0.1 μg/mL of $rLBP_{25}$ had no significant inhibitory effect.

EXAMPLE 16

Effect of rLBP and $rLBP_{25}$ on Bacteria Binding to Monocytes

In this example, the effect of $rLBP_{25}$ on bacterial binding to monocytes and polymorphonuclear neutrophils (PMNs) was compared with that of rLBP. White blood cells (WBC) were isolated by lysis with NH_4Cl of acid-citrate-dextran treated blood and red blood cell ghosts removed by washing. Bacteria were cultured overnight in Trypticase soy broth, washed

extensively, incubated for 1 hour at room temperature with 1 mg/ml fluorescein isothiocyanate (FITC), and washed. Most strains were then incubated for 30 minutes at 60°C and washed again. The concentration of bacterial cells was then determined turbidometrically.

In order to measure bacterial binding and uptake by WBC, WBC adjusted to 10^7/ml in pooled normal human serum (NHS) were mixed with a 5-fold excess of bacteria in NHS and incubated. Experiments were terminated by transferring the tubes to 4°C and/or adding cytochalasin B. Cells were then washed extensively with ice cold DPBS and analyzed immediately on a FACScan (Becton Dickinson, San Jose, CA). Binding is defined as the percent of cells with bacteria at time zero. Uptake is defined as the percent of cells with bacteria after 15 minutes at 37°C minus the percent of cells with bacteria at time zero.

For the determination of the effect of rLBP and $rLBP_{25}$ on bacterial binding to monocytes, isolated WBC in 100% allogeneic human serum mixed with *E. coli* J5 bacteria that had been pretreated with various concentrations of rLBP or $rLBP_{25}$. As shown in Figures 20 and 21, there was minimal binding (< 10%) to either monocytes or PMNs by the bacteria in the absence of rLBP or $rLBP_{25}$. rLBP mediated a concentration-dependent binding of bacteria to monocytes, that reached 30% at 100 μg/mL rLBP (Figure 20). This rLBP-enhanced binding of bacteria to monocytes was shown to be inhibited by an anti-CD14 monoclonal antibody (mAb MY4) but not by an isotype-matched control (mAb 5C2). In striking contrast, $rLBP_{25}$ was unable to mediate such a CD14-dependent enhanced binding to monocytes as induced by rLBP (Figure 21). No comparable increase in binding to PMNs is mediated by either rLBP or $rLBP_{25}$. This

finding is consistent with the much lower levels of CD14 on PMNs as opposed to monocytes.

EXAMPLE 17

LBP Sandwich ELISA

An LBP sandwich ELISA has been developed which utilizes a rabbit anti-rLBP antibody on solid-phase with a biotin-labeled rabbit anti-rLBP as the detector. Both rLBP and $rLBP_{25}$ were detected by this ELISA (Figure 22). The linear range of the rLBP standard curve was 40 to 800 pg/mL.

In this assay, rBPI produced a signal which was approximately 5 orders of magnitude lower than that of rLBP. For example, the signal produced by 100,000 ng/mL of rBPI was equivalent to the signal produced by 0.7 ng/mL of rLBP. Therefore, endogenous BPI will have a negligible effect on the accurate measurement of LBP.

Fifty microliters of affinity purified rabbit anti-rLBP antibody (1 μg/mL in PBS) were incubated overnight at 2-8°C (or alternatively, 1 hour at 37°C) in the wells of Immulon 2 (Dynatech Laboratories Inc., Chantilly, VA) microtiter plates. The antibody solution was removed and 200 μL of 1% non-fat milk in PBS (blocking agent) was added to all wells. After blocking the plates for one hour at room temperature, the wells were washed three times with 300 μL of wash buffer (PBS/0.05% Tween-20). Standards, samples and controls were diluted in triplicate with PBS containing 1% bovine serum albumin, 0.05% Tween 20 (PBS-BSA/Tween) and 10 units/mL of sodium heparin (Sigma Chemical Co., St. Louis, MO) in separate 96-well plates. rLBP or $rLBP_{25}$ standard solutions were prepared as serial two-fold dilutions from 100 to 0.012

ng/mL. Each replicate and dilution of the standards, samples and controls (50 μL) was transferred to the blocked microtiter plates and incubated for one hour at 37°C. After the primary incubation, the wells were washed three times with wash buffer. Biotin-labeled rabbit anti-rLBP antibody was diluted 1/4000 in PBS-BSA/Tween and 50 μL was added to all wells. The plates were then incubated for one hour at 37°C. Subsequently, all wells were washed 3 times with wash buffer. Alkaline phosphatase-labeled streptavidin (Zymed Laboratories Inc., San Francisco, CA) was diluted 1/2000 in PBS-BSA/Tween and 50 μL was added to all wells. After incubation for 15 minutes at 37°C, all wells were washed three times with wash buffer and 3 times with deionized water and the substrate p-nitrophenylphosphate (1 mg/mL in 10% diethanolamine buffer) was added in a volume of 50 μL to all wells. Color development was allowed to proceed for one hour at room temperature, after which 50 μL of 1 N NaOH was added to stop the reaction. The absorbance at 405 nm was determined for all wells using a Vmax Plate Reader (Molecular Devices Corp., Menlo Park, CA).

The mean absorbance at 405 nm (A_{405}) for all samples and standards (in triplicate) were corrected for background by subtracting the mean A_{405} of wells receiving only sample dilute buffer (no BPI) in the primary incubation step. A standard curve was then plotted as A_{405} versus ng/ml of rLBP or $rLBP_{25}$. The linear range was selected, a linear regression analysis was performed and concentrations were determined for samples and controls by interpolation from the standard curve.

EXAMPLE 18

Construction of Vectors for Expression of LBP Derivatives

An LBP derivative was constructed in which the alanine residue at position 131 was mutated to cysteine. This position is analogous to cysteine 132 in the BPI sequence, which appears to be the cysteine involved in homodimer formation. By placing a cysteine in the same position in the LBP sequence, the expressed protein may also have the ability to dimerize via interchain disulfide bond formation through cysteine 131, and the resultant dimer may have increased biological potency as is observed for the BPI dimer. To construct the LBP (1-197) (Cys131) derivative, two complementary oligonucleotides containing the desired mutation were used to replace a portion of the LBP sequence between *Bpm*I and *Pst*I restriction sites, corresponding to the amino acid sequence from residue 127 to residue 133. These oligonucleotides were LBP15: 5'-CCCACAGTCACGTGCTCCAGCTGCA-3'[SEQ ID NO:15], and LBP16: 5'-GCTGGAGCACGTGACTGTGGGCC-3'[SEQ ID NO:16]. These 2 annealed oligonucleotides were ligated to two fragments from pML116: the -409 bp *Hind*III-*Bpm*I fragment and the -3033 bp *Pst*I-*Hind*III fragment, to generate plasmid pML136. Plasmid pML116 is a vector for use in an *in vitro* transcription/translation system encoding LBP (1-197) which corresponds to mammalian vectors pING4505 and pING4508 described above. Plasmid pML136 is then expressed in an *in vitro* transcription/translation system as described above to produce LBP (1-197) (Cys 131). A corresponding vector for expression in mammalian cells can be constructed according to the methods described previously.

EXAMPLE 19

Vectors for Expression of LBP-IgG Hybrid Fusion Proteins

Portions of the LBP protein can be included in fusions with the Fc region of human IgG_1 according to the general methods of co-owned and copending U.S. Patent Application Serial No: 08/064,693 filed May 19, 1993, the disclosure of which is hereby incorporated by reference, which teaches the construction of BPI-IgG fusion proteins. These LBP hybrid proteins may include truncated forms of LBP, such as 1-197, and may also include LBP derivatives containing the alanine 131 to cysteine mutation. These LBP hybrid proteins may be expected to form dimers upon recombinant production and may also have improved pharmacokinetics.

EXAMPLE 20

In Vitro Transcription/Translations of Truncated LBP Fragments and Determination of Their Ability to Mediate LPS Stimulation of TNF Activity

While $rLBP_{25}$ and rLBP interact similarly with lipid A/LPS, rLBP and not $rLBP_{25}$ can stimulate the uptake of LPS and release the TNF by THP-1 cells. These data suggest that the region of LBP responsible for mediation of LPS activity via the CD14 receptor lies between positions 197 and 456 (the end) of the LBP protein sequence. To define this region more precisely, plasmid pML130 containing the entire 456 amino acid sequence of LBP (including silent mutations to introduce a *Cla*I restriction site at position 197-198), was constructed for *in vitro* transcription/translation experiments. Plasmid pML130 corresponds generally to mammalian vector pING4539 described previously. The LBP insert in pML130 is under the

control of the T7 RNA polymerase promoter, which efficiently transcribes RNA from linear DNA templates. Therefore, deletions of the LBP sequence generated via restriction digests of plasmid pML130 in the region between residues 197 and 456 as set out in Table 5 below are used as templates in the *in vitro* transcription/translation reaction to generate truncated LBP protein fragments. The biological activity of these LBP protein fragments are then tested to determine the region of the LBP sequence necessary for mediating the interaction of LPS with the CD14 receptor with the LBP (1-456) holoprotein acting as a positive control for CD14 activity and the $rLBP_{25}$ (1-197) fragment acting as a negative control. Mammalian vectors for expression of the LBP derivatives are produced and the derivatives are expressed according to the methods disclosed previously.

TABLE 5

Restriction enzyme digest	LBP fragment size (amino acids)
*Hind*III	456
*Eco*47III	400
*Bsi*HKAI	342
*Bam*1105I	298
*Bsu*36I	244
*Cla*I	196

EXAMPLE 21

Construction of Vectors for Expression of LBP Hybrid Proteins

A number of vectors were constructed to express LBP hybrid proteins containing both BPI and LBP sequences derived from the amino-terminal domains of each protein. Such proteins are expected to retain LPS binding activity since the LPS binding domain exists within the amino-terminal region of both BPI and LBP. It is further expected that the hybrid proteins comprising amino terminal portions of both LBP and BPI would be unable to mediate LPS effects via interaction with CD14 receptor. Such proteins may further have advantageous biologic or pharmacokinetic properties. Vectors constructed as intermediates or for use in the *in vitro* transcription/translation system have the designation "pIC" or "pML" followed by a number while vectors intended for expression in mammalian cells have the designation "pING" followed by a number.

A vector encoding an LBP hybrid protein combining the elements of pML105 [LBP (1-43)/BPI (44-199)] and pML103 [BPI (1-159)/LBP (158-197)], namely pML134, was constructed to encode LBP (1-43)/BPI (44-159)/LBP (158-197). The construction of pML134 was accomplished by ligating the -458 bp *Nhe*I-*Eco*RI fragment of pML105 to the -3011 bp *Eco*RI-*Nhe*I fragment of pML103. In addition to pING4526 already described, another vector for expression of BPI(1-159)/LBP(158-197) in mammalian cells was constructed which contained optimized elements including Kozak initiation sequence, human kappa poly A/Mouse Kappa genomic transcription termination and CMV promoter as described in coowned and co-pending U.S. Application Serial No. 08/013,801 filed February 2, 1993 the disclosure of which is hereby incorporated by reference. The mammalian vector was designated pING4168.

Two other BPI/LBP hybrids, pML117 and pML118, were constructed by taking advantage of common *Pst*I restriction sites contained in the coding region sequence of both proteins. The starting plasmids for these constructs were pIC127, which contains an insert encoding BPI (1-199), and pIC106, which contains an insert encoding LBP (1-197). pML117, containing an insert encoding BPI (1-137)/LBP (137-197), was constructed by replacing the -200 bp *Pst*I-*Xho*I fragment of BPI in pIC127 with the corresponding LBP fragment from pIC106. pML118, encoding BPI (1-25) LBP (26-135) BPI (137-199) was constructed by replacing an -600 bp *Pst*I-*Pst*I fragment within the BPI coding region of pIC127 with the corresponding LBP fragment from pIC106. The resulting plasmids are used as templates in the *in vitro* transcription/translation reaction method according to Example 7 in order to generate LBP hybrid proteins. Constructs pML103, pML105, pML116, pML117, pML118, pML134 were expressed in the transcription/translation system and the products were all detected by ELISA using appropriate reagents. All the products bound well to Lipid A with the exception of BPI (1-25)/LBP(26-134)/BPI(135-199) which bound poorly. Three products exhibited superior Lipid A binding activity. They were BPI(1-159)/LBP(158-197), BPI(1-135)/LBP(135-197), and LBP(1-43)/BPI(44-159)/LBP(158-197).

Mammalian vectors for expression of the LBP hybrid proteins are produced and the derivatives are expressed according to the methods disclosed previously.

EXAMPLE 22

Construction of Vectors for Expression of LBP Hybrid Proteins Comprising BPI/LBP Active Domain Replacement Mutants

$rBPI_{23}$ possesses three biological activities, LPS binding, heparin binding and bactericidal activity, which have been localized to specific domains of the protein defined by synthetic peptides as disclosed by co-owned and copending U.S. Patent Application Serial No. 08/209,762 filed March 11, 1994 which is a continuation-in-part of U.S. Patent Application No. 08/183,222 filed January 14, 1994, which is a continuation-in-part of U.S Patent Application No. 08/093,292 filed July 15, 1993 which is a continuation-in-part of U.S. Patent Application Serial No. 08/030,644 filed March 12, 1993 the disclosures of which are incorporated herein by reference. Domain I of BPI includes the amino acid sequence of human BPI from about position 17 to about position 45 having the sequence: BPI Domain I ASQQGTAALQKELKRIKPDYSDSFKIKH (SEQ ID NO:17); Domain II of BPI includes the amino acid sequence of human BPI from about position 65 to about position 99 having the sequence BPI Domain II SSQISMVPNVGLKFSISNANIKISGKWKAQKRFLK (SEQ ID NO:18) while Domain III of BPI includes the amino acid sequence of human BPI from about position 142 to about position 169 having the sequence BPI Domain III VHVHISKSKVGWLIQLFHKKIESALRNK (SEQ ID NO:19) The corresponding domain I of LBP includes the amino acid sequence of human LBP from about position 17 to about position 45 having the sequence LBP Domain I AAQEGLLALQSELLRITLPDFTGDLRIPH (SEQ IS NO:20); Domain II of LBP includes the amino acid sequence of human LBP from about position 65 to about position 99 having the sequence LBP Domain II

HSALRPVPGQGLSLSISDSSIRVQGRWKVRKSFFK (SEQ ID NO:21); while Domain III of LBP includes the amino acid sequence of human LBP from about position 141 to about position 167 having the sequence LBP Domain III VEVDMSGDLGWLLNLFHNQIESKFQKV (SEQ ID NO 22).

Several plasmids encoding LBP hybrid proteins were constructed for *in vitro* transcription/translation studies in which sequences selected from Domains II and/or III of BPI were inserted to replace the corresponding region of LBP_{25}. Conversely, other plasmids were constructed in which sequences selected from Domains II and/or III of LBP were inserted to replace sequences from the corresponding region of BPI_{23}.

First, plasmid pML131 was constructed comprising silent mutations in Domain II of BPI_{23}. Specifically, the nucleotide sequence between *Afl*II and *Bsr*BI restriction sites in the BPI_{23} coding region of pIC127 was replaced with the annealed oligonucleotides BPI-66: 5'-TTAAATTTTCGATATCCAACGCCAATATTAAGATCTCCGGAAAATG GAAGGCACAAAAGCGCTTCCTTAAGATGAG-3' (SEQ ID NO:23) and BPI-67: 5'-CTCATCTTAAGGAAGCGCTTTTGTGCCTTCCATTTTCCGGAGATCT TAATATTGGCGTTGGATATCGAAAAT (SEQ ID NO:24). This replacement changed the nucleotide sequence but not the amino acid sequence in the region between residues 77 and 100, and introduced additional restriction sites which could be used for site-directed mutagenesis (*Eco*RV, *Ssp*I, *Bgl*II, *Bsp*EI, *Eco*47III, and relocated *Afl*II).

In addition plasmid pML140 was constructed comprising silent mutations in Domain III of BPI_{23}. Specifically, the nucleotide sequence between *Pml*I and *Bst*BI restriction sites in the BPI_{23} coding

region of pIC127 was replaced with the annealed oligonucleotides BPI-78: 5'-
GTGCACATTTCGAAGAGCAAAGTGGGGTGGCTGATCCAATTGTTC
CACAAAAAAATTGAGAGCGCGCTG-3' (SEQ ID NO:25) and BPI-79: 5'-
CGCAGCGCGCTCTCAATTTTTTTGTGGAACAATTGGATCAGCCACC
CCACTTTGCTCTTCGAAATGTGCAC-3' (SEQ ID NO:26). This replacement changed the nucleotide sequence but not the amino acid sequence in the region between residues 146 and 163, and introduced additional restriction sites which could be used for site-directed mutagenesis (relocated *Bst*BI, *Mun*I, and *Bss*HII).

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [LBP(1-87)/BPI (88-100)/LBP(101-197)] was constructed in which a portion of Domain II of BPI replaced the corresponding LBP sequence. The hybrid protein comprised the first 87 amino acid residues of LBP, amino acid residues 88-100 of BPI and amino acid residues 101-197 of LBP. The nucleotide sequence between *Ava*II and *Ban*II restriction sites in the LBP_{25} coding region of pML116 was replaced with the annealed oligonucleotides BPI-74: 5'-
GTCAGCGGGAAATGGAAGGCACAAAAGAGATTTTTAAAAATGCAG
GGCT-3' (SEQ ID NO:27) and BPI-75: 5'-
CTGCATTTTTAAAAATCTCTTTTGTGCCTTCCATTTCCCGCT-3' (SEQ ID NO:28). This replacement essentially changed the amino acid sequence of residues 88-100 of LBP_{25} from QGRWKVRKSFFKL (SEQ ID NO:29) to SGKWKAQKRFLKM (SEQ ID NO:30). The resulting plasmid was designated pML135.

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [LBP(1-146)/BPI (148-161)/LBP(160-197)] was constructed in which a portion of Domain III of BPI replaced the corresponding LBP sequence. The hybrid protein comprised the first 146 amino acid residues of LBP, amino acid residues 148-161 of BPI and amino acid residues 160-197 of LBP. Specifically, the nucleotide sequence between *AFI*III and *Sca*I restriction sites in the LBP_{25} coding region of pML116 was replaced with the annealed oligonucleotides BPI-76: 5'-CATGTCGAAGAGCAAAGTGGGGTGGCTGATCCAACTCTTCCACAA AAAAATTGAGTCCAAATTTCAGAAAGT-3'(SEQ ID NO:31) and BPI-77: 5'-ACTTTCTGAAATTTGGACTCAATTTTTTTGTGGAAGAGTTGGATCA GCCACCCCACTTTGCTCTTCGA-3'(SEQ ID NO:32). This replacement essentially changed the amino acid sequence of residues 147-159 of LBP_{25} from GDLGWLLNLFHNQ (SEQ ID NO:33) to the corresponding BPI sequence KSKVGWLIQLFHKK (SEQ ID NO:34). This plasmid was designated pML137.

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [LBP(1-87)/BPI(88-100)/LBP(101-146)/BPI(148-161)/LBP(160-197)] was constructed in which portions of Domains II and III of BPI replaced the corresponding LBP sequences. The hybrid protein comprised the first 87 amino acid residues of LBP, amino acid residues 88-100 of BPI, amino acid residues 101-146 of LBP, amino acid residues 148-161 of BPI and amino acid residues 160-197 of LBP. Plasmid pML138 was constructed from pML135 and pML137 by replacing an ~292 bp *Ban*II-*Xho*I fragment of the coding region of pML135 with the

corresponding fragment from pML137 to introduce the domain III mutations into the plasmid already containing the domain II mutations.

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [BPI(1-85)/LBP(86-99)/BPI(100-199)] was constructed in which a portion of Domain II of LBP replaced the corresponding BPI sequence. The hybrid protein comprised the first 85 amino acid residues of BPI, amino acid residues 86-99 of LBP and amino acid residues 100-199 of BPI. Specifically, the nucleotide sequence between *Ssp*I and *Afl*II restriction sites in the BPI_{23} coding region of pML131 was replaced with the annealed oligonucleotides BPI-80: 5'-ATTCGTGTACAGGGCAGGTGGAAGGTGCGCAAGTCATTCT-3' (SEQ ID NO:35) and BPI-81: 5'-TTAAAGAATGACTTGCGCACCTTCCACCTGCCCTGTACACGAAT-3' (SEQ ID NO:36). This replacement essentially changed the amino acid sequence of residues 86-99 of BPI_{23} from KISGKWKAQKRFLK (SEQ ID NO:37) to the corresponding LBP sequence RVQGRWKVRKSFFK. This plasmid was designated pML141 (SEQ ID NO:38).

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [BPI(1-147/LBP(147-159)/BPI(162-199)] was constructed in which a portion of Domain III of LBP replaced the corresponding BPI sequence. The hybrid protein comprised the first 147 amino acid residues of BPI, amino acid residues 147-159 of LBP and amino acid residues 162-199 of BPI. The nucleotide sequence between *Bst*BI and *Bss*HII restriction sites in the BPI_{23} coding region of pML140 was replaced with the annealed oligonucleotides BPI-82: 5'-CGGGAGACTTGGGGTGGCTGTTGAACCTCTTCCACAACCAGATTG AGAG-3' (SEQ ID NO:39) and BPI-83: 5'-

CGCGCTCTCAATCTGGTTGTGGAAGAGGTTCAACAGCCACCCCAA GTCTCC-3' (SEQ ID NO:40). This replacement essentially changed the amino acid sequence of residues 148-161 of BPI_{23} from KSKVGWLIQLFHKK (SEQ ID NO:41) to the corresponding LBP sequence GDLGWLLNLFHNQ (SEQ ID NO:42). This plasmid was designated pML142.

A plasmid for *in vitro* transcription/translation studies encoding the LBP hybrid protein [BPI(1-85)/LBP(86-99)/BPI(100-147)/LBP(147-159)/BPI(162-199)] was constructed in which portions of Domains II and III of LBP replaced the corresponding BPI sequences. The hybrid protein comprised the first 85 amino acid residues of BPI, amino acid residues 86-99 of LBP, amino acid residues 100-147 of BPI, amino acid residues 147-159 of LBP and amino acid residues 162-199 of BPI. pML143 can be constructed from pML141 and pML142 by replacing an ~175 bp *PmlI-XhoI* fragment in the coding region of pML141 with the corresponding fragment from pML142 to introduce the domain III mutations into the plasmid already containing the domain II mutations. This plasmid was designated pML143.

The resulting plasmids are used as templates in the *in vitro* transcription/translation reaction to generate LBP hybrid proteins. Mammalian vectors for expression of the LBP hybrid proteins are produced and the derivatives are expressed according to the methods disclosed previously. The biological activities of these LBP hybrid proteins are then tested according to the methods set out above.

EXAMPLE 23

LBP derivatives in the form of synthetic LBP peptides were prepared according to the methods of Merrifield, *J. Am, Chem, Soc. 85*: 2149 (1963) and Merrifield, *Anal Chem. 38*: 1905-1914 (1966) using an Applied Biosystems, Inc. Model 432 synthesizer. The resulting derivatives comprised portions of the LBP sequence corresponding to either of BPI Domain II or III. The LBP derivative designated LBP-1 consisted of residues 73 through 99 of LBP having the sequence GQGLSLSISDSSIRVQGRWKVRKSFFK (SEQ ID NO:43). The LBP derivative designated LBP-2 consisted of residues 140 through 161 of LBP and had the sequence DVEVDMSGDSGWLLNLFHNQIE (SEQ ID NO:44). The LBP derivatives were subjected to an *Limulus* Amoebocyte Lysate (LAL) assay using a quantiative chromogenic LAL kit (Whitaker Bioproducts, Inc., Walkersville, MD) to determine neutralization of LPS. LBP-1 was found to neutralize endotoxin in the LAL assay while LBP-2 did not.

Numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon consideration of the foregoing description of the presently preferred embodiments thereof. Consequently, the only limitations which should be placed upon the scope of the present invention are those which appear in the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: XOMA CORPORATION
(B) STREET: 2910 Seventh Street
(C) CITY: Berkeley
(D) STATE: California
(E) COUNTRY: United States of America
(F) POSTAL CODE: 94710

(ii) TITLE OF INVENTION: Lipopolysaccharide Binding Protein Derivatives

(iii) NUMBER OF SEQUENCES:44

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Marshall, O'Toole, Gerstein, Murray & Borun
(B) STREET: 6300 Sears Tower, 233 South Wacker Drive
(C) CITY: Chicago
(D) STATE: Illinois
(E) COUNTRY: United States of America
(F) ZIP: 60606-6402

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Smith, Jeffrey S.
(B) REGISTRATION NUMBER: 31,879
(C) REFERENCE/DOCKET NUMBER: 27129/32137

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312/474-6300
(B) TELEFAX: 312/474-0448
(C) TELEX: 25-3856

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..591

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "$rLBP_{25}$"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GCC AAC CCC GGC TTG GTC GCC AGG ATC ACC GAC AAG GGA CTG CAG TAT        48
Ala Asn Pro Gly Leu Val Ala Arg Ile Thr Asp Lys Gly Leu Gln Tyr
  1               5                  10                  15

GCG GCC CAG GAG GGG CTA TTG GCT CTG CAG AGT GAG CTG CTC AGG ATC        96
Ala Ala Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg Ile
            20                  25                  30

ACG CTG CCT GAC TTC ACC GGG GAC TTG AGG ATC CCC CAC GTC GGC CGT       144
Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly Arg
        35                  40                  45

GGG CGC TAT GAG TTC CAC AGC CTG AAC ATC CAC AGC TGT GAG CTG CTT       192
Gly Arg Tyr Glu Phe His Ser Leu Asn Ile His Ser Cys Glu Leu Leu
    50                  55                  60

CAC TCT GCG CTG AGG CCT GTC CCT GGC CAG GGC CTG AGT CTC AGC ATC       240
His Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser Ile
65                  70                  75                  80

TCC GAC TCC TCC ATC CGG GTC CAG GGC AGG TGG AAG GTG CGC AAG TCA       288
Ser Asp Ser Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser
                85                  90                  95

TTC TTC AAA CTA CAG GGC TCC TTT GAT GTC AGT GTC AAG GGC ATC AGC       336
Phe Phe Lys Leu Gln Gly Ser Phe Asp Val Ser Val Lys Gly Ile Ser
            100                 105                 110

ATT TCG GTC AAC CTC CTG TTG GGC AGC GAG TCC TCC GGG AGG CCC ACA       384
Ile Ser Val Asn Leu Leu Leu Gly Ser Glu Ser Ser Gly Arg Pro Thr
        115                 120                 125

GTT ACT GCC TCC AGC TGC AGC AGT GAC ATC GCT GAC GTG GAG GTG GAC       432
Val Thr Ala Ser Ser Cys Ser Ser Asp Ile Ala Asp Val Glu Val Asp
    130                 135                 140

ATG TCG GGA GAC TTG GGG TGG CTG TTG AAC CTC TTC CAC AAC CAG ATT       480
Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln Ile
145                 150                 155                 160

GAG TCC AAG TTC CAG AAA GTA CTG GAG AGC AGG ATT TGC GAA ATG ATC       528
Glu Ser Lys Phe Gln Lys Val Leu Glu Ser Arg Ile Cys Glu Met Ile
```

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                165                 170                 175

CAG AAA TCG GTG TCC TCC GAT CTA CAG CCT TAT CTC CAA ACT CTG CCA      576
Gln Lys Ser Val Ser Ser Asp Leu Gln Pro Tyr Leu Gln Thr Leu Pro
            180                 185                 190

GTT ACA ACA GAG ATT                                              591
Val Thr Thr Glu Ile
        195
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 197 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: protein
(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "$rLBP_{25}$"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Ala Asn Pro Gly Leu Val Ala Arg Ile Thr Asp Lys Gly Leu Gln Tyr
  1               5                  10                  15

Ala Ala Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg Ile
             20                  25                  30

Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly Arg
         35                  40                  45

Gly Arg Tyr Glu Phe His Ser Leu Asn Ile His Ser Cys Glu Leu Leu
     50                  55                  60

His Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser Ile
 65                  70                  75                  80

Ser Asp Ser Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser
                 85                  90                  95

Phe Phe Lys Leu Gln Gly Ser Phe Asp Val Ser Val Lys Gly Ile Ser
            100                 105                 110

Ile Ser Val Asn Leu Leu Leu Gly Ser Glu Ser Ser Gly Arg Pro Thr
        115                 120                 125

Val Thr Ala Ser Ser Cys Ser Ser Asp Ile Ala Asp Val Glu Val Asp
    130                 135                 140

Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln Ile
145                 150                 155                 160
```

```
Glu Ser Lys Phe Gln Lys Val Leu Glu Ser Arg Ile Cys Glu Met Ile
                165                 170                 175

Gln Lys Ser Val Ser Ser Asp Leu Gln Pro Tyr Leu Gln Thr Leu Pro
            180                 185                 190

Val Thr Thr Glu Ile
        195
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1443 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1443

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 76..1443

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "rLBP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATG GGG GCC TTG GCC AGA GCC CTG CCG TCC ATA CTG CTG GCA TTG CTG        48
Met Gly Ala Leu Ala Arg Ala Leu Pro Ser Ile Leu Leu Ala Leu Leu
-25                 -20                 -15                 -10

CTT ACG TCC ACC CCA GAG GCT CTG GGT GCC AAC CCC GGC TTG GTC GCC        96
Leu Thr Ser Thr Pro Glu Ala Leu Gly Ala Asn Pro Gly Leu Val Ala
                -5                  1                   5

AGG ATC ACC GAC AAG GGA CTG CAG TAT GCG GCC CAG GAG GGG CTA TTG       144
Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu
        10                  15                  20

GCT CTG CAG AGT GAG CTG CTC AGG ATC ACG CTG CCT GAC TTC ACC GGG       192
Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly
    25                  30                  35

GAC TTG AGG ATC CCC CAC GTC GGC CGT GGG CGC TAT GAG TTC CAC AGC       240
Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His Ser
40                  45                  50                  55
```

```
CTG AAC ATC CAC AGC TGT GAG CTG CTT CAC TCT GCG CTG AGG CCT GTC        288
Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val
                60                  65                  70

CCT GGC CAG GGC CTG AGT CTC AGC ATC TCC GAC TCC TCC ATC CGG GTC        336
Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val
            75                  80                  85

CAG GGC AGG TGG AAG GTG CGC AAG TCA TTC TTC AAA CTA CAG GGC TCC        384
Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser
        90                  95                  100

TTT GAT GTC AGT GTC AAG GGC ATC AGC ATT TCG GTC AAC CTC CTG TTG        432
Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu
    105                 110                 115

GGC AGC GAG TCC TCC GGG AGG CCC ACA GTT ACT GCC TCC AGC TGC AGC        480
Gly Ser Glu Ser Ser Gly Arg Pro Thr Val Thr Ala Ser Ser Cys Ser
120                 125                 130                 135

AGT GAC ATC GCT GAC GTG GAG GTG GAC ATG TCG GGA GAC TTG GGG TGG        528
Ser Asp Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp
                140                 145                 150

CTG TTG AAC CTC TTC CAC AAC CAG ATT GAG TCC AAG TTC CAG AAA GTA        576
Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val
            155                 160                 165

CTG GAG AGC AGG ATT TGC GAA ATG ATC CAG AAA TCG GTG TCC TCC GAT        624
Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp
        170                 175                 180

CTA CAG CCT TAT CTC CAA ACT CTG CCA GTT ACA ACA GAG ATT GAC AGT        672
Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser
    185                 190                 195

TTC GCC GAC ATT GAT TAT AGC TTA GTG GAA GCC CCT CGG GCA ACA GCC        720
Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala
200                 205                 210                 215

CAG ATG CTG GAG GTG ATG TTT AAG GGT GAA ATC TTT CAT CGT AAC CAC        768
Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe His Arg Asn His
                220                 225                 230

CGT TCT CCA GTT ACC CTC CTT GCT GCA GTC ATG AGC CTT CCT GAG GAA        816
Arg Ser Pro Val Thr Leu Leu Ala Ala Val Met Ser Leu Pro Glu Glu
            235                 240                 245

CAC AAC AAA ATG GTC TAC TTT GCC ATC TCG GAT TAT GTC TTC AAC ACG        864
His Asn Lys Met Val Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr
        250                 255                 260

GCC AGC CTG GTT TAT CAT GAG GAA GGA TAT CTG AAC TTC TCC ATC ACA        912
Ala Ser Leu Val Tyr His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr
    265                 270                 275
```

```
GAT GAG ATG ATA CCG CCT GAC TCT AAT ATC CGA CTG ACC ACC AAG TCC      960
Asp Glu Met Ile Pro Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser
280                 285                 290                 295

TTC CGA CCC TTC GTC CCA CGG TTA GCC AGG CTC TAC CCC AAC ATG AAC     1008
Phe Arg Pro Phe Val Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn
                300                 305                 310

CTG GAA CTC CAG GGA TCA GTG CCC TCT GCT CCG CTC CTG AAC TTC AGC     1056
Leu Glu Leu Gln Gly Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser
            315                 320                 325

CCT GGG AAT CTG TCT GTG GAC CCC TAT ATG GAG ATA GAT GCC TTT GTG     1104
Pro Gly Asn Leu Ser Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val
        330                 335                 340

CTC CTG CCC AGC TCC AGC AAG GAG CCT GTC TTC CGG CTC AGT GTG GCC     1152
Leu Leu Pro Ser Ser Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala
    345                 350                 355

ACT AAT GTG TCC GCC ACC TTG ACC TTC AAT ACC AGC AAG ATC ACT GGG     1200
Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly
360                 365                 370                 375

TTC CTG AAG CCA GGA AAG GTA AAA GTG GAA CTG AAA GAA TCC AAA GTT     1248
Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val
                380                 385                 390

GGA CTA TTC AAT GCA GAG CTG TTG GAA GCG CTC CTC AAC TAT TAC ATC     1296
Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile
            395                 400                 405

CTT AAC ACC TTC TAC CCC AAG TTC AAT GAT AAG TTG GCC GAA GGC TTC     1344
Leu Asn Thr Phe Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe
        410                 415                 420

CCC CTT CCT CTG CTG AAG CGT GTT CAG CTC TAC GAC CTT GGG CTG CAG     1392
Pro Leu Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln
    425                 430                 435

ATC CAT AAG GAC TTC CTG TTC TTG GGT GCC AAT GTC CAA TAC ATG AGA     1440
Ile His Lys Asp Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg
440                 445                 450                 455

GTT                                                                 1443
Val
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 481 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "rLBP"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Gly Ala Leu Ala Arg Ala Leu Pro Ser Ile Leu Leu Ala Leu Leu
-25                 -20                 -15                 -10

Leu Thr Ser Thr Pro Glu Ala Leu Gly Ala Asn Pro Gly Leu Val Ala
                -5                   1                   5

Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu
        10                  15                  20

Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly
    25                  30                  35

Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His Ser
40                  45                  50                  55

Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val
                60                  65                  70

Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val
            75                  80                  85

Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser
        90                  95                  100

Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu
    105                 110                 115

Gly Ser Glu Ser Ser Gly Arg Pro Thr Val Thr Ala Ser Ser Cys Ser
120                 125                 130                 135

Ser Asp Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp
                140                 145                 150

Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val
            155                 160                 165

Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val Ser Ser Asp
        170                 175                 180

Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile Asp Ser
    185                 190                 195

Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu Ala Pro Arg Ala Thr Ala
200                 205                 210                 215

Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe His Arg Asn His
```

```
                220                 225                 230

Arg Ser Pro Val Thr Leu Leu Ala Ala Val Met Ser Leu Pro Glu Glu
            235                 240                 245

His Asn Lys Met Val Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr
        250                 255                 260

Ala Ser Leu Val Tyr His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr
    265                 270                 275

Asp Glu Met Ile Pro Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser
280                 285                 290                 295

Phe Arg Pro Phe Val Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn
                300                 305                 310

Leu Glu Leu Gln Gly Ser Val Pro Ser Ala Pro Leu Leu Asn Phe Ser
            315                 320                 325

Pro Gly Asn Leu Ser Val Asp Pro Tyr Met Glu Ile Asp Ala Phe Val
        330                 335                 340

Leu Leu Pro Ser Ser Ser Lys Glu Pro Val Phe Arg Leu Ser Val Ala
    345                 350                 355

Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser Lys Ile Thr Gly
360                 365                 370                 375

Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser Lys Val
                380                 385                 390

Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile
            395                 400                 405

Leu Asn Thr Phe Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe
        410                 415                 420

Pro Leu Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln
    425                 430                 435

Ile His Lys Asp Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg
440                 445                 450                 455

Val
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP-Bsm"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATGCAGCC AACCCCGGCT TGGTCGCCA 29

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP-2"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CTCGAGCTAA ATCTCTGTTG TAACTGGC 28

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP-3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CATGTCGACA CCATGGGGGC CTTG 24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP-4"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CATGCCGCGG TCAAACTCTC ATGTA 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP 241-245"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTCATGAGCC TTCCT 15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP 241-245"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Val Met Ser Leu Pro
1 5

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1813 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 31..1491

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 124..1491

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
CAGGCCTTGA GGTTTTGGCA GCTCTGGAGG ATG AGA GAG AAC ATG GCC AGG GGC       54
                                 Met Arg Glu Asn Met Ala Arg Gly
                                 -31 -30                 -25

CCT TGC AAC GCG CCG AGA TGG GTG TCC CTG ATG GTG CTC GTC GCC ATA      102
Pro Cys Asn Ala Pro Arg Trp Val Ser Leu Met Val Leu Val Ala Ile
           -20                 -15                 -10

GGC ACC GCC GTG ACA GCG GCC GTC AAC CCT GGC GTC GTG GTC AGG ATC      150
Gly Thr Ala Val Thr Ala Ala Val Asn Pro Gly Val Val Val Arg Ile
        -5                   1                   5

TCC CAG AAG GGC CTG GAC TAC GCC AGC CAG CAG GGG ACG GCC GCT CTG      198
Ser Gln Lys Gly Leu Asp Tyr Ala Ser Gln Gln Gly Thr Ala Ala Leu
 10                  15                  20                  25

CAG AAG GAG CTG AAG AGG ATC AAG ATT CCT GAC TAC TCA GAC AGC TTT      246
Gln Lys Glu Leu Lys Arg Ile Lys Ile Pro Asp Tyr Ser Asp Ser Phe
                30                  35                  40

AAG ATC AAG CAT CTT GGG AAG GGG CAT TAT AGC TTC TAC AGC ATG GAC      294
Lys Ile Lys His Leu Gly Lys Gly His Tyr Ser Phe Tyr Ser Met Asp
            45                  50                  55

ATC CGT GAA TTC CAG CTT CCC AGT TCC CAG ATA AGC ATG GTG CCC AAT      342
Ile Arg Glu Phe Gln Leu Pro Ser Ser Gln Ile Ser Met Val Pro Asn
        60                  65                  70

GTG GGC CTT AAG TTC TCC ATC AGC AAC GCC AAT ATC AAG ATC AGC GGG      390
Val Gly Leu Lys Phe Ser Ile Ser Asn Ala Asn Ile Lys Ile Ser Gly
    75                  80                  85
```

```
AAA TGG AAG GCA CAA AAG AGA TTC TTA AAA ATG AGC GGC AAT TTT GAC      438
Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Met Ser Gly Asn Phe Asp
 90                  95                  100                 105

CTG AGC ATA GAA GGC ATG TCC ATT TCG GCT GAT CTG AAG CTG GGC AGT      486
Leu Ser Ile Glu Gly Met Ser Ile Ser Ala Asp Leu Lys Leu Gly Ser
                110                 115                 120

AAC CCC ACG TCA GGC AAG CCC ACC ATC ACC TGC TCC AGC TGC AGC AGC      534
Asn Pro Thr Ser Gly Lys Pro Thr Ile Thr Cys Ser Ser Cys Ser Ser
            125                 130                 135

CAC ATC AAC AGT GTC CAC GTG CAC ATC TCA AAG AGC AAA GTC GGG TGG      582
His Ile Asn Ser Val His Val His Ile Ser Lys Ser Lys Val Gly Trp
        140                 145                 150

CTG ATC CAA CTC TTC CAC AAA AAA ATT GAG TCT GCG CTT CGA AAC AAG      630
Leu Ile Gln Leu Phe His Lys Lys Ile Glu Ser Ala Leu Arg Asn Lys
    155                 160                 165

ATG AAC AGC CAG GTC TGC GAG AAA GTG ACC AAT TCT GTA TCC TCC AAG      678
Met Asn Ser Gln Val Cys Glu Lys Val Thr Asn Ser Val Ser Ser Lys
170                 175                 180                 185

CTG CAA CCT TAT TTC CAG ACT CTG CCA GTA ATG ACC AAA ATA GAT TCT      726
Leu Gln Pro Tyr Phe Gln Thr Leu Pro Val Met Thr Lys Ile Asp Ser
                190                 195                 200

GTG GCT GGA ATC AAC TAT GGT CTG GTG GCA CCT CCA GCA ACC ACG GCT      774
Val Ala Gly Ile Asn Tyr Gly Leu Val Ala Pro Pro Ala Thr Thr Ala
            205                 210                 215

GAG ACC CTG GAT GTA CAG ATG AAG GGG GAG TTT TAC AGT GAG AAC CAC      822
Glu Thr Leu Asp Val Gln Met Lys Gly Glu Phe Tyr Ser Glu Asn His
        220                 225                 230

CAC AAT CCA CCT CCC TTT GCT CCA CCA GTG ATG GAG TTT CCC GCT GCC      870
His Asn Pro Pro Pro Phe Ala Pro Pro Val Met Glu Phe Pro Ala Ala
    235                 240                 245

CAT GAC CGC ATG GTA TAC CTG GGC CTC TCA GAC TAC TTC TTC AAC ACA      918
His Asp Arg Met Val Tyr Leu Gly Leu Ser Asp Tyr Phe Phe Asn Thr
250                 255                 260                 265

GCC GGG CTT GTA TAC CAA GAG GCT GGG GTC TTG AAG ATG ACC CTT AGA      966
Ala Gly Leu Val Tyr Gln Glu Ala Gly Val Leu Lys Met Thr Leu Arg
                270                 275                 280

GAT GAC ATG ATT CCA AAG GAG TCC AAA TTT CGA CTG ACA ACC AAG TTC     1014
Asp Asp Met Ile Pro Lys Glu Ser Lys Phe Arg Leu Thr Thr Lys Phe
            285                 290                 295

TTT GGA ACC TTC CTA CCT GAG GTG GCC AAG AAG TTT CCC AAC ATG AAG     1062
Phe Gly Thr Phe Leu Pro Glu Val Ala Lys Lys Phe Pro Asn Met Lys
        300                 305                 310
```

```
ATA CAG ATC CAT GTC TCA GCC TCC ACC CCG CCA CAC CTG TCT GTG CAG      1110
Ile Gln Ile His Val Ser Ala Ser Thr Pro Pro His Leu Ser Val Gln
    315                 320                 325

CCC ACC GGC CTT ACC TTC TAC CCT GCC GTG GAT GTC CAG GCC TTT GCC      1158
Pro Thr Gly Leu Thr Phe Tyr Pro Ala Val Asp Val Gln Ala Phe Ala
330                 335                 340                 345

GTC CTC CCC AAC TCC TCC CTG GCT TCC CTC TTC CTG ATT GGC ATG CAC      1206
Val Leu Pro Asn Ser Ser Leu Ala Ser Leu Phe Leu Ile Gly Met His
                350                 355                 360

ACA ACT GGT TCC ATG GAG GTC AGC GCC GAG TCC AAC AGG CTT GTT GGA      1254
Thr Thr Gly Ser Met Glu Val Ser Ala Glu Ser Asn Arg Leu Val Gly
            365                 370                 375

GAG CTC AAG CTG GAT AGG CTG CTC CTG GAA CTG AAG CAC TCA AAT ATT      1302
Glu Leu Lys Leu Asp Arg Leu Leu Leu Glu Leu Lys His Ser Asn Ile
        380                 385                 390

GGC CCC TTC CCG GTT GAA TTG CTG CAG GAT ATC ATG AAC TAC ATT GTA      1350
Gly Pro Phe Pro Val Glu Leu Leu Gln Asp Ile Met Asn Tyr Ile Val
    395                 400                 405

CCC ATT CTT GTG CTG CCC AGG GTT AAC GAG AAA CTA CAG AAA GGC TTC      1398
Pro Ile Leu Val Leu Pro Arg Val Asn Glu Lys Leu Gln Lys Gly Phe
410                 415                 420                 425

CCT CTC CCG ACG CCG GCC AGA GTC CAG CTC TAC AAC GTA GTG CTT CAG      1446
Pro Leu Pro Thr Pro Ala Arg Val Gln Leu Tyr Asn Val Val Leu Gln
                430                 435                 440

CCT CAC CAG AAC TTC CTG CTG TTC GGT GCA GAC GTT GTC TAT AAA          1491
Pro His Gln Asn Phe Leu Leu Phe Gly Ala Asp Val Val Tyr Lys
            445                 450                 455

TGAAGGCACC AGGGGTGCCG GGGGCTGTCA GCCGCACCTG TTCCTGATGG GCTGTGGGGC    1551

ACCGGCTGCC TTTCCCCAGG GAATCCTCTC CAGATCTTAA CCAAGAGCCC CTTGCAAACT    1611

TCTTCGACTC AGATTCAGAA ATGATCTAAA CACGAGGAAA CATTATTCAT TGGAAAAGTG    1671

CATGGTGTGT ATTTTAGGGA TTATGAGCTT CTTTCAAGGG CTAAGGCTGC AGAGATATTT    1731

CCTCCAGGAA TCGTGTTTCA ATTGTAACCA AGAAATTTCC ATTTGTGCTT CATGAAAAAA    1791

AACTTCTGGT TTTTTTCATG TG                                              1813
```

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 487 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Arg Glu Asn Met Ala Arg Gly Pro Cys Asn Ala Pro Arg Trp Val
-31 -30                 -25                 -20

Ser Leu Met Val Leu Val Ala Ile Gly Thr Ala Val Thr Ala Ala Val
-15                 -10                  -5                   1

Asn Pro Gly Val Val Val Arg Ile Ser Gln Lys Gly Leu Asp Tyr Ala
              5                   10                  15

Ser Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile Lys
         20                  25                  30

Ile Pro Asp Tyr Ser Asp Ser Phe Lys Ile Lys His Leu Gly Lys Gly
     35                  40                  45

His Tyr Ser Phe Tyr Ser Met Asp Ile Arg Glu Phe Gln Leu Pro Ser
 50                  55                  60                  65

Ser Gln Ile Ser Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile Ser
                70                  75                  80

Asn Ala Asn Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe
             85                  90                  95

Leu Lys Met Ser Gly Asn Phe Asp Leu Ser Ile Glu Gly Met Ser Ile
        100                 105                 110

Ser Ala Asp Leu Lys Leu Gly Ser Asn Pro Thr Ser Gly Lys Pro Thr
    115                 120                 125

Ile Thr Cys Ser Ser Cys Ser Ser His Ile Asn Ser Val His Val His
130                 135                 140                 145

Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
                150                 155                 160

Ile Glu Ser Ala Leu Arg Asn Lys Met Asn Ser Gln Val Cys Glu Lys
            165                 170                 175

Val Thr Asn Ser Val Ser Ser Lys Leu Gln Pro Tyr Phe Gln Thr Leu
        180                 185                 190

Pro Val Met Thr Lys Ile Asp Ser Val Ala Gly Ile Asn Tyr Gly Leu
    195                 200                 205

Val Ala Pro Pro Ala Thr Thr Ala Glu Thr Leu Asp Val Gln Met Lys
210                 215                 220                 225
```

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Gly Glu Phe Tyr Ser Glu Asn His His Asn Pro Pro Pro Phe Ala Pro
                230                 235                 240

Pro Val Met Glu Phe Pro Ala Ala His Asp Arg Met Val Tyr Leu Gly
            245                 250                 255

Leu Ser Asp Tyr Phe Phe Asn Thr Ala Gly Leu Val Tyr Gln Glu Ala
        260                 265                 270

Gly Val Leu Lys Met Thr Leu Arg Asp Asp Met Ile Pro Lys Glu Ser
    275                 280                 285

Lys Phe Arg Leu Thr Thr Lys Phe Phe Gly Thr Phe Leu Pro Glu Val
290                 295                 300                 305

Ala Lys Lys Phe Pro Asn Met Lys Ile Gln Ile His Val Ser Ala Ser
                310                 315                 320

Thr Pro Pro His Leu Ser Val Gln Pro Thr Gly Leu Thr Phe Tyr Pro
            325                 330                 335

Ala Val Asp Val Gln Ala Phe Ala Val Leu Pro Asn Ser Ser Leu Ala
        340                 345                 350

Ser Leu Phe Leu Ile Gly Met His Thr Thr Gly Ser Met Glu Val Ser
    355                 360                 365

Ala Glu Ser Asn Arg Leu Val Gly Glu Leu Lys Leu Asp Arg Leu Leu
370                 375                 380                 385

Leu Glu Leu Lys His Ser Asn Ile Gly Pro Phe Pro Val Glu Leu Leu
                390                 395                 400

Gln Asp Ile Met Asn Tyr Ile Val Pro Ile Leu Val Leu Pro Arg Val
            405                 410                 415

Asn Glu Lys Leu Gln Lys Gly Phe Pro Leu Pro Thr Pro Ala Arg Val
        420                 425                 430

Gln Leu Tyr Asn Val Val Leu Gln Pro His Gln Asn Phe Leu Leu Phe
    435                 440                 445

Gly Ala Asp Val Val Tyr Lys
450                 455
```

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI-18"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAGCATCTTG GGAAGGGG 18

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI-11"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TATTTTGGTC ATTACTGGCA GAGT 24

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP15"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CCCACAGTCA CGTGCTCCAG CTGCA 25

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP16"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCTGGAGCAC GTGACTGTGG GCC 23

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI Domain I"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Ala Ser Gln Gln Gly Thr Ala Ala Leu Gln Lys Glu Leu Lys Arg Ile
1 5 10 15

Lys Pro Asp Tyr Ser Asp Ser Phe Lys Ile Lys His
20 25

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI Domain II"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Ser Gln Ile Ser Met Val Pro Asn Val Gly Leu Lys Phe Ser Ile
1 5 10 15

Ser Asn Ala Asn Ile Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg
20 25 30

Phe Leu Lys
35

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "BPI Domain III"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Val His Val His Ile Ser Lys Ser Lys Val Gly Trp Leu Ile Gln Leu
1 5 10 15

Phe His Lys Lys Ile Glu Ser Ala Leu Arg Asn Lys
20 25

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP Domain I"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Ala Ala Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg Ile
1 5 10 15

Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His
20 25

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP Domain II"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

His Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser Ile
1 5 10 15

Ser Asp Ser Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser
20 25 30

Phe Phe Lys
35

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
(A) NAME/KEY: misc_feature

(D) OTHER INFORMATION: "LBP Domain III"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe
1 5 10 15

His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val
20 25

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 76 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TTAAATTTTC GATATCCAAC GCCAATATTA AGATCTCCGG AAAATGGAAG GCACAAAAGC 60

GCTTCCTTAA GATGAG 76

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTCATCTTAA GGAAGCGCTT TTGTGCCTTC CATTTTCCGG AGATCTTAAT ATTGGCGTTG 60

GATATCGAAA AT 72

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 69 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GTGCACATTT CGAAGAGCAA AGTGGGGTGG CTGATCCAAT TGTTCCACAA AAAAATTGAG 60

AGCGCGCTG 69

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 71 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

CGCAGCGCGC TCTCAATTTT TTTGTGGAAC AATTGGATCA GCCACCCCAC TTTGCTCTTC 60

GAAATGTGCA C 71

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTCAGCGGGA AATGGAAGGC ACAAAAGAGA TTTTTAAAAA TGCAGGGCT 49

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CTGCATTTTT AAAAATCTCT TTTGTGCCTT CCATTTCCCG CT 42

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys Met
1 5 10

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 72 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CATGTCGAAG AGCAAAGTGG GGTGGCTGAT CCAACTCTTC CACAAAAAAA TTGAGTCCAA 60

ATTTCAGAAA GT 72

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

ACTTTCTGAA ATTTGGACTC AATTTTTTTG TGGAAGAGTT GGATCAGCCA CCCCACTTTG 60

CTCTTCGA 68

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

ATTCGTGTAC AGGGCAGGTG GAAGGTGCGC AAGTCATTCT 40

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 44 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TTAAAGAATG ACTTGCGCAC CTTCCACCTG CCCTGTACAC GAAT 44

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Lys Ile Ser Gly Lys Trp Lys Ala Gln Lys Arg Phe Leu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 49 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CGGGAGACTT GGGGTGGCTG TTGAACCTCT TCCACAACCA GATTGAGAG 49

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

CGCGCTCTCA ATCTGGTTGT GGAAGAGGTT CAACAGCCAC CCCAAGTCTC C 51

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Lys Ser Lys Val Gly Trp Leu Ile Gln Leu Phe His Lys Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val Gln
1 5 10 15

Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys
20 25

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Asp Val Glu Val Asp Met Ser Gly Asp Ser Gly Trp Leu Leu Asn Leu
1 5 10 15

Phe His Asn Gln Ile Glu
20

WHAT IS CLAIMED IS:

1. An LBP protein derivative having an ability to bind to LPS and lacking CD14-mediated immunostimulatory properties.

2. The derivative according to claim 1 which has a molecular weight of about 25 kD.

3. The derivative according to claim 1 consisting of a portion of the amino-terminal half of LBP.

4. The derivative according to claim 3 consisting of amino acids 1 through 197 of SEQ ID NO:1.

5. The derivative according to claim 1 which is LBP (1-197) (Cys131).

6. An LBP derivative hybrid protein having an ability to bind to LPS and lacking CD14-mediated immunostimulatory properties and characterized by the presence of at least a portion of an LPS binding domain of BPI selected from the group consisting of: ASQQGTAALQKELKRIKPDYSDSFKIKH (SEQ ID NO:17); SSQISMVPNVGLKFSISNANIKISGKWKAQKRFLK (SEQ ID NO:18); and VHVHISKSKVGWLIQLFHKKIESALRNK (SEQ ID NO:19).

7. The hybrid protein according to claim 6 characterized by the presence of at least a portion of an LPS binding domain of LBP selected from the group consisting of:
AAQEGLLALQSELLRITLPDFTGDLRIPH (SEQ IS NO:20);
HSALRPVPGQGLSLSISDSSIRVQGRWKVRKSFFK (SEQ ID NO:21); and
VEVDMSGDLGWLLNLFHNQIESKFQKV (SEQ ID NO 22).

8. The hybrid protein according to claim 6 consisting of a portion of the amino-terminal half of LBP/and a portion of the amino-terminal half of BPI.

9. The hybrid protein according to claim 6 which is selected from the group consisting of LBP(1-43)/BPI(44-199), BPI(1-159)/LBP(158-197), LBP(1-43)/BPI(44-159)/LBP(158-197), BPI(1-137)/LBP(137-197), BPI(1-25)/LBP(26-135)/BPI(137-199), [LBP(1-87)/BPI(88-100)/LBP(101-197)], [LBP(1-146)/BPI(148-161)/LBP(160-197)], [LBP(1-87)/BPI(88-100)/LBP(101-146)/BPI(148-161)/LBP(160-197)], [BPI(1-85)/LBP(86-99)/BPI(100-199)], [BPI(1-147/LBP(147-159)/BPI(162-199)], and [BPI(1-85)/LBP(86-99)/BPI(100-147)/LBP(147-159)/BPI(162-199)].

10. The LBP derivative hybrid protein having an ability to bind LPS and lacking CD-14 mediated immunostimulatory properties which is an LBP/IgG fusion protein.

11. A pharmaceutical composition comprising an LBP protein derivative or LBP derivative hybrid protein according claim 1, 6 or 10 and a pharmaceutically acceptable diluent, adjuvant or carrier.

12. A DNA sequence encoding an LBP derivative or LBP derivative hybrid or protein according to claim 1, 6 or 10.

13. A DNA vector comprising the DNA sequence according to claim 12.

14. A host cell stably transformed or transfected with a DNA sequence according to claim 12 in a manner allowing expression in the host cell of the protein encoded thereby.

15. A method of treating a gram-negative bacterial infection and the sequelae thereof comprising administering an LBP protein derivative or LBP derivative hybrid protein according to claim 1, 6 or 10.

16. The method of claim 15 wherein the LBP protein derivative or LBP derivative hybrid protein is administered at a dosage of from about 0.1 mg/kg to about 100 mg/kg of body weight.

17. The use of an LBP protein derivative or an LBP derivative hybrid protein claim 1, 6 or 10 in the manufacture of a medicament for the treatment of gram negative bacterial infection and the sequelae thereof.

LBP25 Sequence

```
  1 GCC AAC CCC GGC TTG GTC GCC AGG ATC ACC GAC AAG GGA CTG CAG TAT GCG GCC
  1 Ala Asn Pro Gly Leu Val Ala Arg Ile Thr Asp Lys Gly Leu Gln Tyr Ala Ala

 55 CAG GAG GGG CTA TTG GCT CTG CAG AGT GAG CTG CTC AGG ATC ACG CTG CCT GAC
 19 Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu Leu Arg Ile Thr Leu Pro Asp

109 TTC ACC GGG GAC TTG AGG ATC CCC CAC GTC GGC CGT GGG CGC TAT GAG TTC CAC
 37 Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly Arg Gly Arg Tyr Glu Phe His

163 AGC CTG AAC ATC CAC AGC TGT GAG CTG CTT CAC TCT GCG CTG AGG CCT GTC CCT
 55 Ser Leu Asn Ile His Ser Cys Glu Leu Leu His Ser Ala Leu Arg Pro Val Pro

217 GGC CAG GGC CTG AGT CTC AGC ATC TCC GAC TCC TCC ATC CGG GTC CAG GGC AGG
 73 Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser Ser Ile Arg Val Gln Gly Arg

271 TGG AAG GTG CGC AAG TCA TTC TTC AAA CTA CAG GGC TCC TTT GAT GTC AGT GTC
 91 Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln Gly Ser Phe Asp Val Ser Val

325 AAG GGC ATC AGC ATT TCG GTC AAC CTC CTG TTG GGC AGC GAG TCC TCC GGG AGG
109 Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu Gly Ser Glu Ser Ser Gly Arg

379 CCC ACA GTT ACT GCC TCC AGC TGC AGC AGT GAC ATC GCT GAC GTG GAG GTG GAC
127 Pro Thr Val Thr Ala Ser Ser Cys Ser Ser Asp Ile Ala Asp Val Glu Val Asp

433 ATG TCG GGA GAC TTG GGG TGG CTG TTG AAC CTC TTC CAC AAC CAG ATT GAG TCC
145 Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu Phe His Asn Gln Ile Glu Ser

487 AAG TTC CAG AAA GTA CTG GAG AGC AGG ATT TGC GAA ATG ATC CAG AAA TCG GTG
163 Lys Phe Gln Lys Val Leu Glu Ser Arg Ile Cys Glu Met Ile Gln Lys Ser Val

541 TCC TCC GAT CTA CAG CCT TAT CTC CAA ACT CTG CCA GTT ACA ACA GAG ATT
181 Ser Ser Asp Leu Gln Pro Tyr Leu Gln Thr Leu Pro Val Thr Thr Glu Ile
```

FIGURE 1

LBP Sequence

```
1 ATG GGG GCC TTG GCC AGA GCC CTG CCG TCC ATA CTG CTG GCA TTG CTG CTT ACG

55 TCC ACC CCA GAG GCT CTG GGT GCC AAC CCC GGC TTG GTC GCC AGG ATC ACC GAC
                              1▶Ala Asn Pro Gly Leu Val Ala Arg Ile Thr Asp

109 AAG GGA CTG CAG TAT GCG GCC CAG GAG GGG CTA TTG GCT CTG CAG AGT GAG CTG
 12▶Lys Gly Leu Gln Tyr Ala Ala Gln Glu Gly Leu Leu Ala Leu Gln Ser Glu Leu

163 CTC AGG ATC ACG CTG CCT GAC TTC ACC GGG GAC TTG AGG ATC CCC CAC GTC GGC
 30▶Leu Arg Ile Thr Leu Pro Asp Phe Thr Gly Asp Leu Arg Ile Pro His Val Gly

217 CGT GGG CGC TAT GAG TTC CAC AGC CTG AAC ATC CAC AGC TGT GAG CTG CTT CAC
 48▶Arg Gly Arg Tyr Glu Phe His Ser Leu Asn Ile His Ser Cys Glu Leu Leu His

271 TCT GCG CTG AGG CCT GTC CCT GGC CAG GGC CTG AGT CTC AGC ATC TCC GAC TCC
 66▶Ser Ala Leu Arg Pro Val Pro Gly Gln Gly Leu Ser Leu Ser Ile Ser Asp Ser

325 TCC ATC CGG GTC CAG GGC AGG TGG AAG GTG CGC AAG TCA TTC TTC AAA CTA CAG
 84▶Ser Ile Arg Val Gln Gly Arg Trp Lys Val Arg Lys Ser Phe Phe Lys Leu Gln

379 GGC TCC TTT GAT GTC AGT GTC AAG GGC ATC AGC ATT TCG GTC AAC CTC CTG TTG
102▶Gly Ser Phe Asp Val Ser Val Lys Gly Ile Ser Ile Ser Val Asn Leu Leu Leu

433 GGC AGC GAG TCC TCC GGG AGG CCC ACA GTT ACT GCC TCC AGC TGC AGC AGT GAC
120▶Gly Ser Glu Ser Ser Gly Arg Pro Thr Val Thr Ala Ser Ser Cys Ser Ser Asp

487 ATC GCT GAC GTG GAG GTG GAC ATG TCG GGA GAC TTG GGG TGG CTG TTG AAC CTC
138▶Ile Ala Asp Val Glu Val Asp Met Ser Gly Asp Leu Gly Trp Leu Leu Asn Leu

541 TTC CAC AAC CAG ATT GAG TCC AAG TTC CAG AAA GTA CTG GAG AGC AGG ATT TGC
156▶Phe His Asn Gln Ile Glu Ser Lys Phe Gln Lys Val Leu Glu Ser Arg Ile Cys

595 GAA ATG ATC CAG AAA TCG GTG TCC TCC GAT CTA CAG CCT TAT CTC CAA ACT CTG
174▶Glu Met Ile Gln Lys Ser Val Ser Ser Asp Leu Gln Pro Tyr Leu Gln Thr Leu

649 CCA GTT ACA ACA GAG ATT GAC AGT TTC GCC GAC ATT GAT TAT AGC TTA GTG GAA
192▶Pro Val Thr Thr Glu Ile Asp Ser Phe Ala Asp Ile Asp Tyr Ser Leu Val Glu

703 GCC CCT CGG GCA ACA GCC CAG ATG CTG GAG GTG ATG TTT AAG GGT GAA ATC TTT
210▶Ala Pro Arg Ala Thr Ala Gln Met Leu Glu Val Met Phe Lys Gly Glu Ile Phe

757 CAT CGT AAC CAC CGT TCT CCA GTT ACC CTC CTT GCT GCA GTC ATG AGC CTT CCT
228▶His Arg Asn His Arg Ser Pro Val Thr Leu Leu Ala Ala Val Met Ser Leu Pro

811 GAG GAA CAC AAC AAA ATG GTC TAC TTT GCC ATC TCG GAT TAT GTC TTC AAC ACG
246▶Glu Glu His Asn Lys Met Val Tyr Phe Ala Ile Ser Asp Tyr Val Phe Asn Thr
```

FIGURE 2A

```
 865 GCC AGC CTG GTT TAT CAT GAG GAA GGA TAT CTG AAC TTC TCC ATC ACA GAT GAC
 264▶Ala Ser Leu Val Tyr His Glu Glu Gly Tyr Leu Asn Phe Ser Ile Thr Asp Asp

 919 ATG ATA CCG CCT GAC TCT AAT ATC CGA CTG ACC ACC AAG TCC TTC CGA CCC TTC
 282▶Met Ile Pro Pro Asp Ser Asn Ile Arg Leu Thr Thr Lys Ser Phe Arg Pro Phe

 973 GTC CCA CGG TTA GCC AGG CTC TAC CCC AAC ATG AAC CTG GAA CTC CAG GGA TCA
 300▶Val Pro Arg Leu Ala Arg Leu Tyr Pro Asn Met Asn Leu Glu Leu Gln Gly Ser

1027 GTG CCC TCT GCT CCG CTC CTG AAC TTC AGC CCT GGG AAT CTG TCT GTG GAC CCC
 318▶Val Pro Ser Ala Pro Leu Leu Asn Phe Ser Pro Gly Asn Leu Ser Val Asp Pro

1081 TAT ATG GAG ATA GAT GCC TTT GTG CTC CTG CCC AGC TCC AGC AAG GAG CCT GTC
 336▶Tyr Met Glu Ile Asp Ala Phe Val Leu Leu Pro Ser Ser Ser Lys Glu Pro Val

1135 TTC CGG CTC AGT GTG GCC ACT AAT GTG TCC GCC ACC TTG ACC TTC AAT ACC AGC
 354▶Phe Arg Leu Ser Val Ala Thr Asn Val Ser Ala Thr Leu Thr Phe Asn Thr Ser

1189 AAG ATC ACT GGG TTC CTG AAG CCA GGA AAG GTA AAA GTG GAA CTG AAA GAA TCC
 372▶Lys Ile Thr Gly Phe Leu Lys Pro Gly Lys Val Lys Val Glu Leu Lys Glu Ser

1243 AAA GTT GGA CTA TTC AAT GCA GAG CTG TTG GAA GCG CTC CTC AAC TAT TAC ATC
 390▶Lys Val Gly Leu Phe Asn Ala Glu Leu Leu Glu Ala Leu Leu Asn Tyr Tyr Ile

1297 CTT AAC ACC TTC TAC CCC AAG TTC AAT GAT AAG TTG GCC GAA GGC TTC CCC CTT
 408▶Leu Asn Thr Phe Tyr Pro Lys Phe Asn Asp Lys Leu Ala Glu Gly Phe Pro Leu

1351 CCT CTG CTG AAG CGT GTT CAG CTC TAC GAC CTT GGG CTG CAG ATC CAT AAG GAC
 426▶Pro Leu Leu Lys Arg Val Gln Leu Tyr Asp Leu Gly Leu Gln Ile His Lys Asp

1405 TTC CTG TTC TTG GGT GCC AAT GTC CAA TAC ATG AGA GTT
 444▶Phe Leu Phe Leu Gly Ala Asn Val Gln Tyr Met Arg Val
```

FIGURE 2B

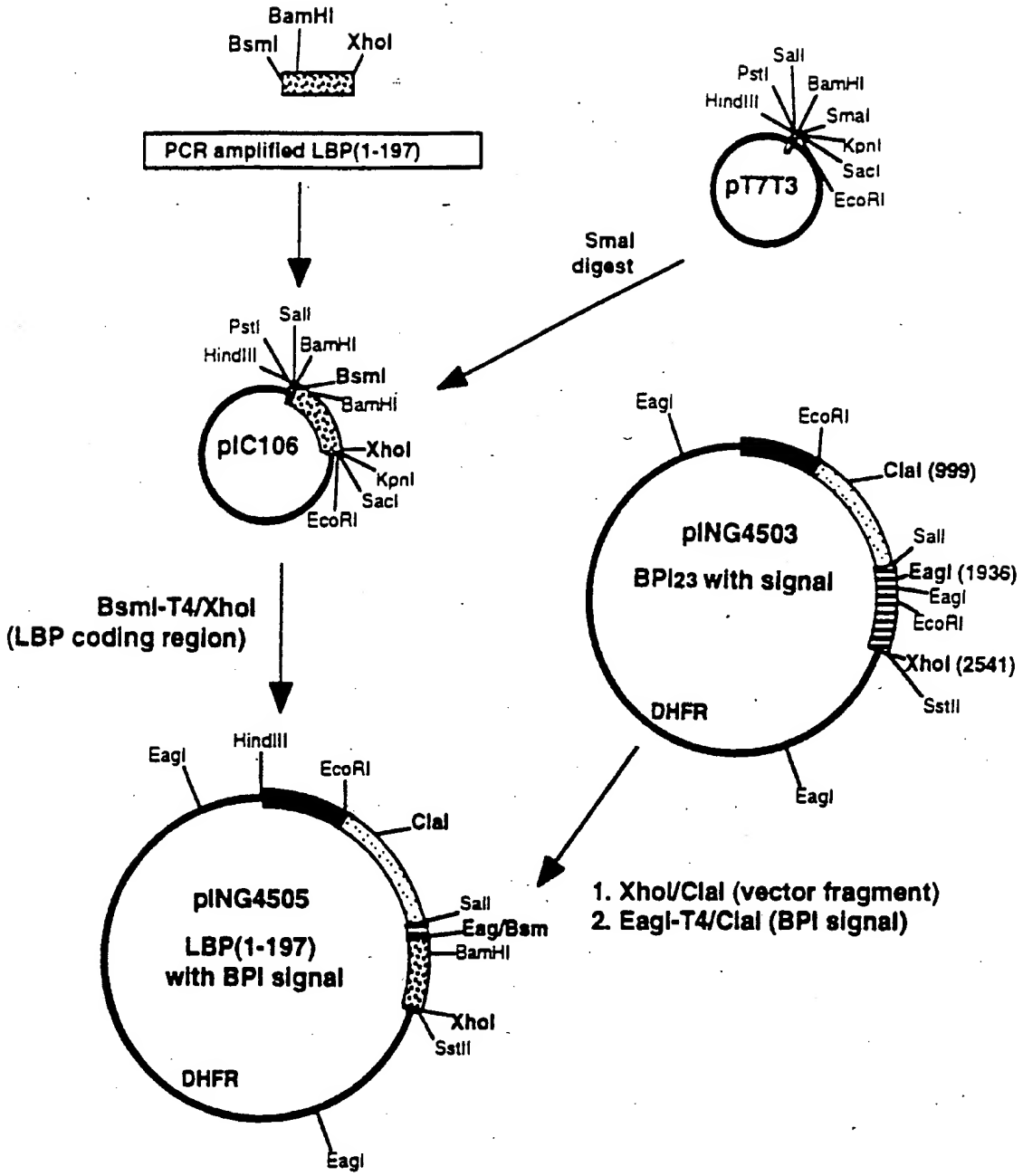

BamHI
BsmI
XhoI
PCR amplified LBP(1-197)
SalI
PstI
BamHI
HindIII
SmaI
KpnI
SacI
EcoRI
pT7T3
SmaI digest
pIC106
BsmI-T4/XhoI (LBP coding region)
EagI
EcoRI
ClaI (999)
pING4503
BPI23 with signal
EagI (1936)
XhoI (2541)
SstII
DHFR
HindIII
pING4505
LBP(1-197) with BPI signal
Eag/Bsm
1. XhoI/ClaI (vector fragment)
2. EagI-T4/ClaI (BPI signal)

FIGURE 3

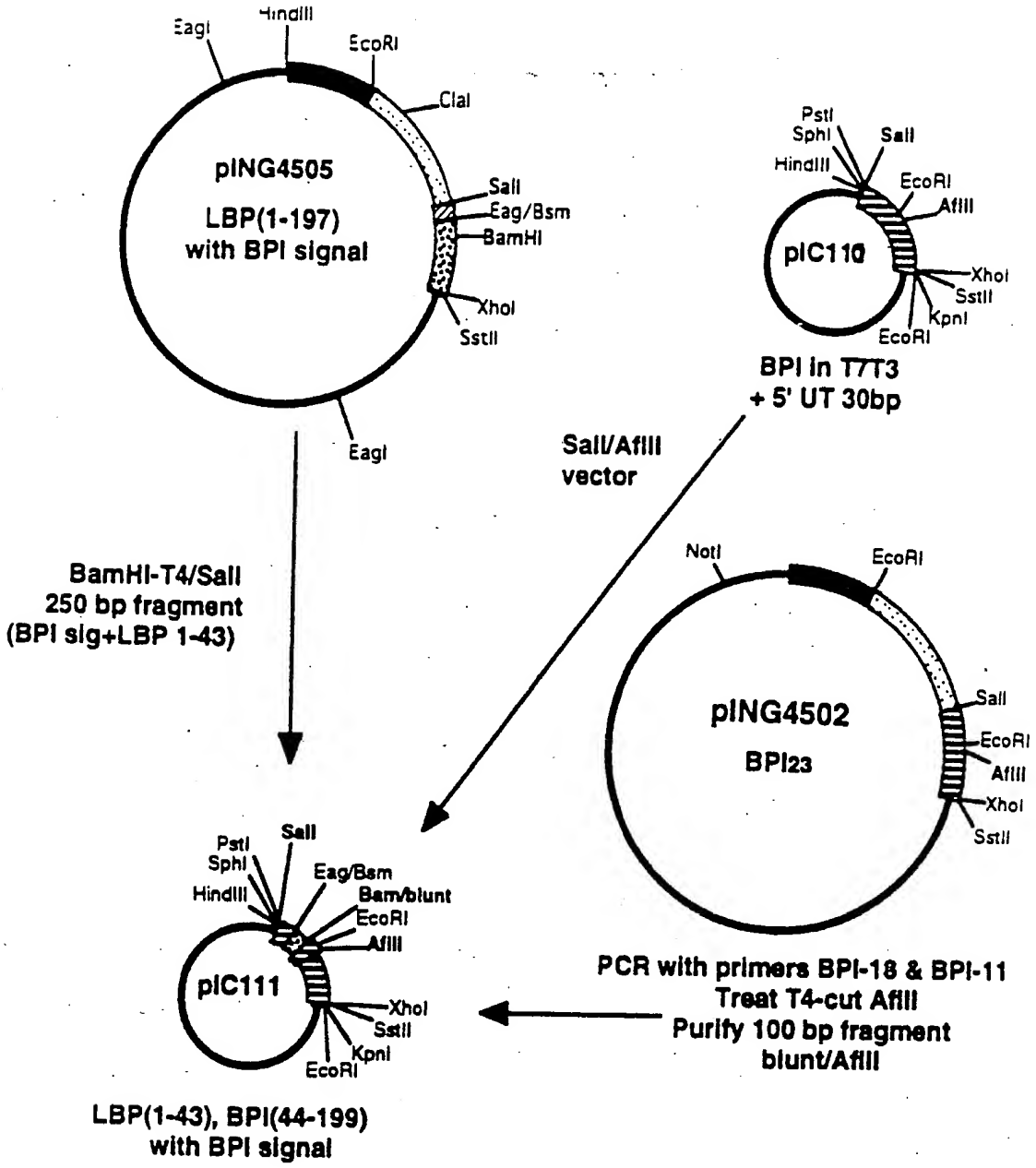

EagI
HindIII
EcoRI
ClaI
pING4505
LBP(1-197)
with BPI signal
SalI
Eag/Bsm
BamHI
XhoI
SstII
EagI
PstI
SphI
SalI
HindIII
EcoRI
AflII
pIC110
XhoI
SstII
KpnI
EcoRI
BPI in T7T3
+ 5' UT 30bp
SalI/AflII
vector
BamHI-T4/SalI
250 bp fragment
(BPI sig+LBP 1-43)
NotI
EcoRI
pING4502
BPI23
SalI
EcoRI
AflII
XhoI
SstII
PstI
SphI
HindIII
SalI
Eag/Bsm
Bam/blunt
EcoRI
AflII
pIC111
XhoI
SstII
KpnI
EcoRI
PCR with primers BPI-18 & BPI-11
Treat T4-cut AflII
Purify 100 bp fragment
blunt/AflII
LBP(1-43), BPI(44-199)
with BPI signal

FIGURE 4

FIGURE 5

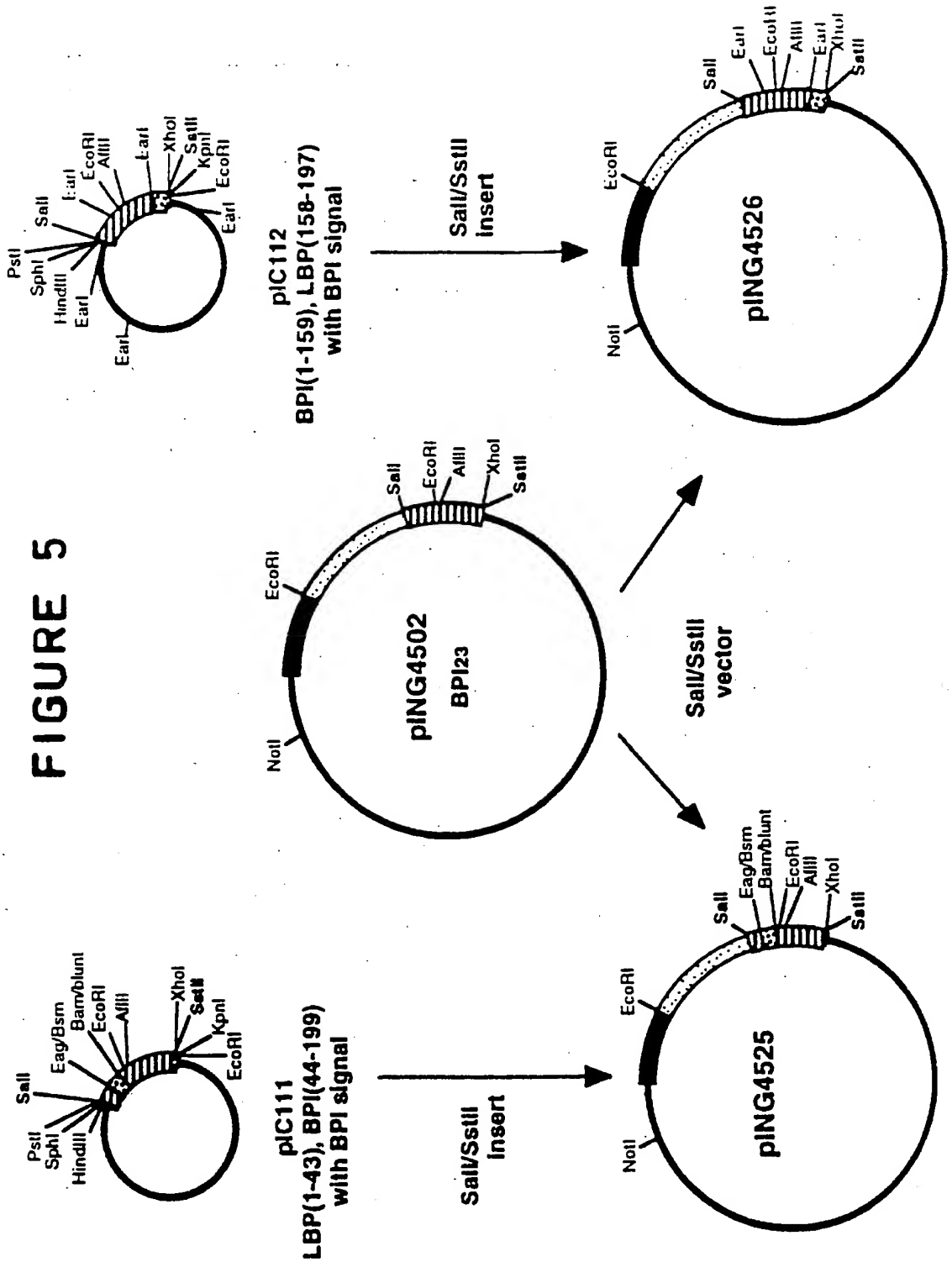
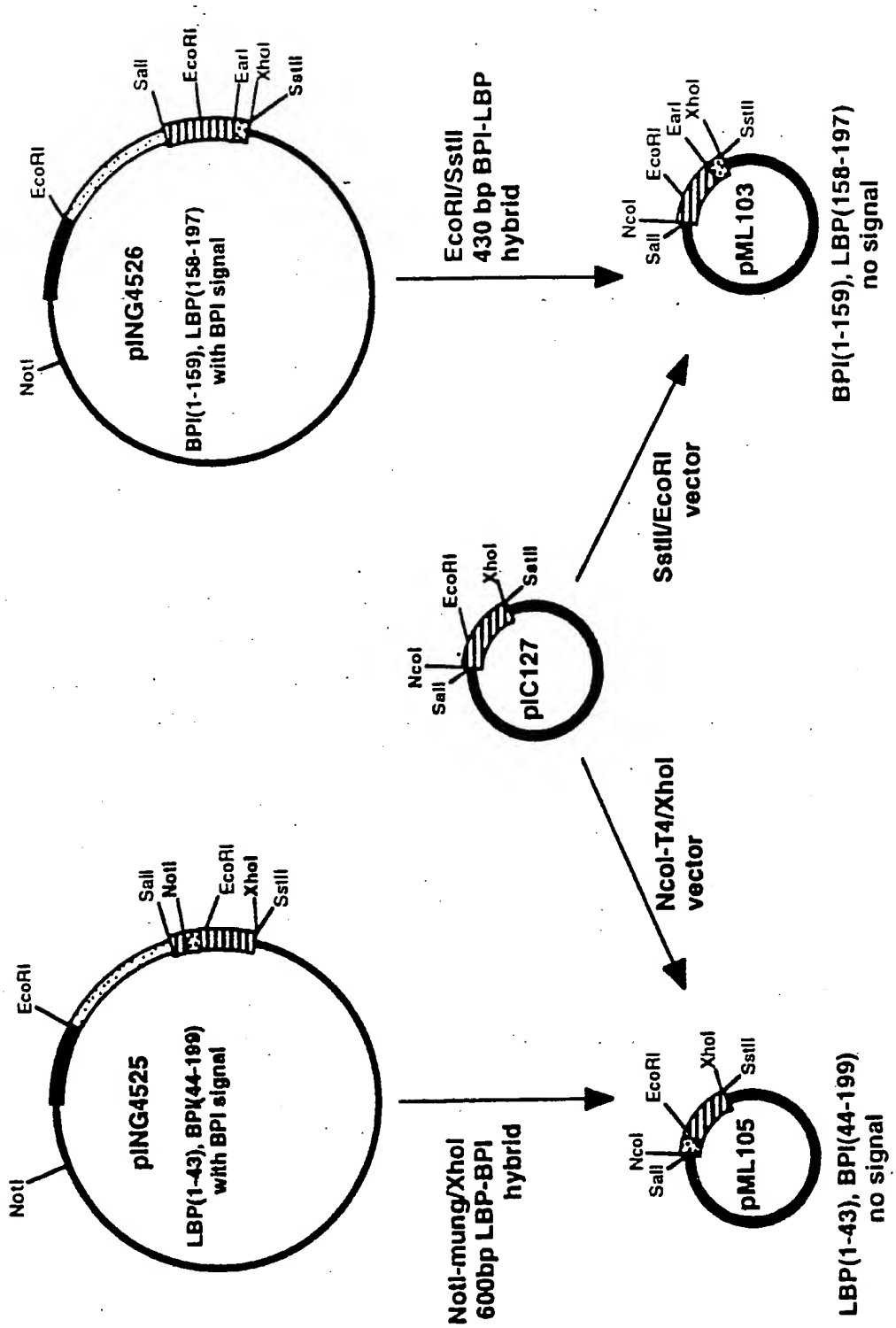

pIC112
BPI(1-159), LBP(158-197)
with BPI signal
Sall/SstII
insert
pING4526
pING4502
BPI23
Sall/SstII
vector
pIC111
LBP(1-43), BPI(44-199)
with BPI signal
Sall/SstII
insert
pING4525

FIGURE 6


pING4526
BPI(1-159), LBP(158-197)
with BPI signal
EcoRI
NotI
SalI
EcoRI
EarI
XhoI
SstII
EcoRI/SstII
430 bp BPI-LBP
hybrid
pML103
NcoI
SalI
EcoRI
EarI
XhoI
SstII
BPI(1-159), LBP(158-197)
no signal
SstII/EcoRI
vector
pIC127
NcoI
SalI
EcoRI
XhoI
SstII
NcoI-T4/XhoI
vector
pING4525
LBP(1-43), BPI(44-199)
with BPI signal
NotI
EcoRI
SalI
NotI
EcoRI
XhoI
SstII
NotI-mung/XhoI
600bp LBP-BPI
hybrid
pML105
NcoI
SalI
EcoRI
XhoI
SstII
LBP(1-43), BPI(44-199)
no signal

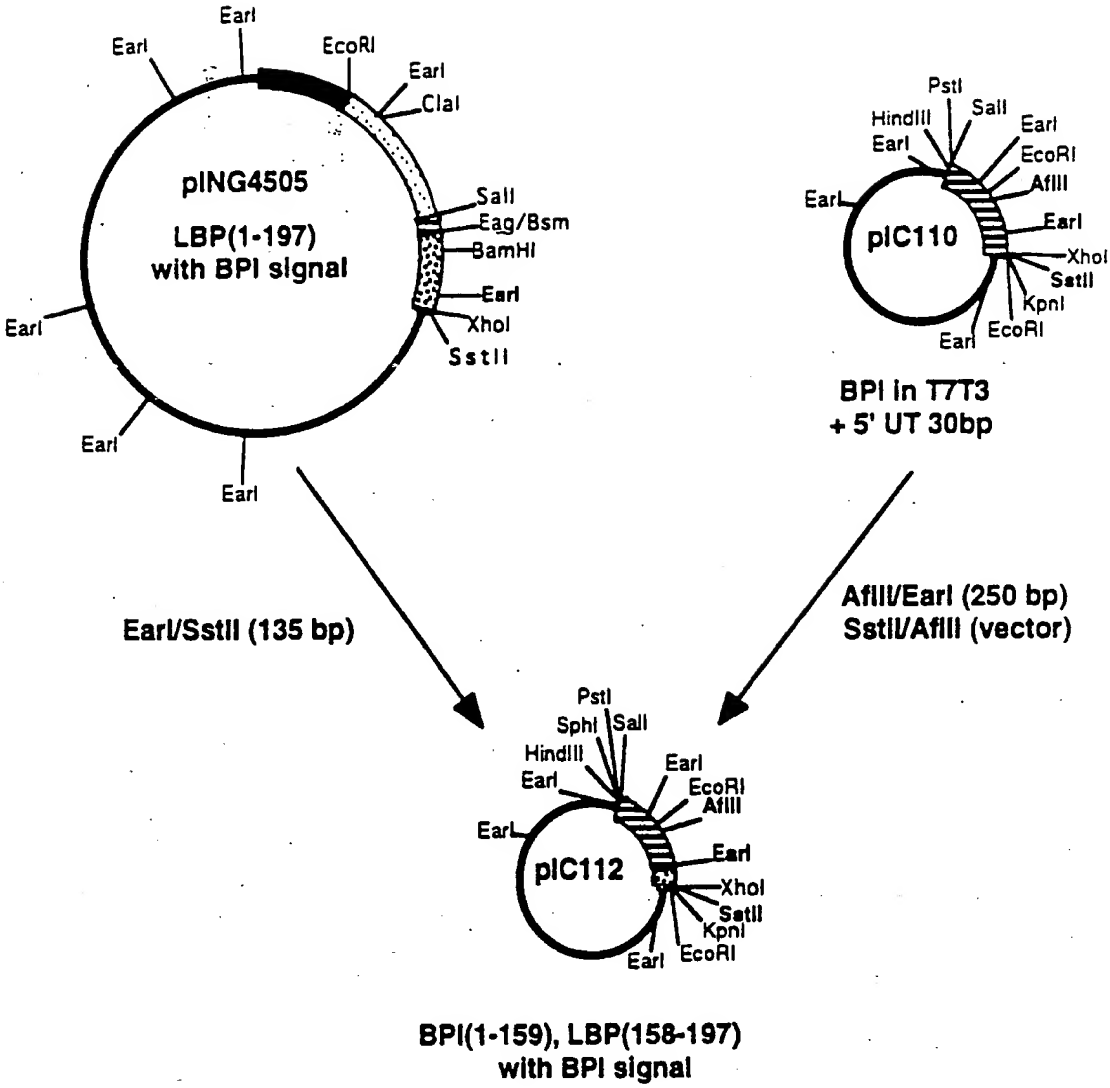
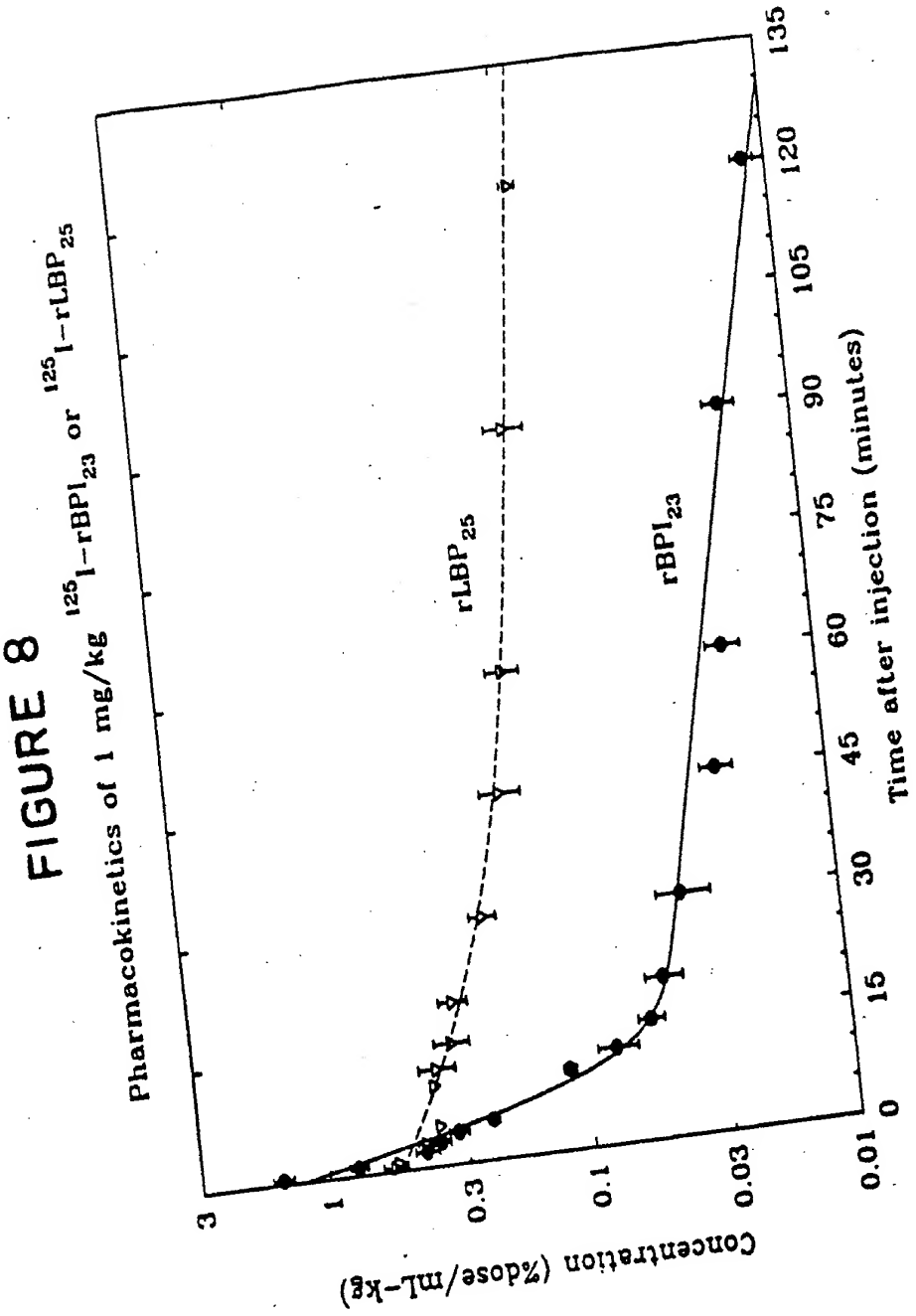

pING4505
LBP(1-197)
with BPI signal
EarI
EcoRI
ClaI
SalI
Eag/Bsm
BamHI
XhoI
SstII
pIC110
PstI
HindIII
SalI
AflII
KpnI
BPI in T7T3
+ 5' UT 30bp
EarI/SstII (135 bp)
AflII/EarI (250 bp)
SstII/AflII (vector)
SphI
pIC112
BPI(1-159), LBP(158-197)
with BPI signal

FIGURE 7

FIGURE 8


Pharmacokinetics of 1 mg/kg ^{125}I-rBPI$_{23}$ or ^{125}I-rLBP$_{25}$
rLBP$_{25}$
rBPI$_{23}$
Concentration (%dose/mL-kg)
3
1
0.3
0.1
0.03
0.01
Time after injection (minutes)
0
15
30
45
60
75
90
105
120
135

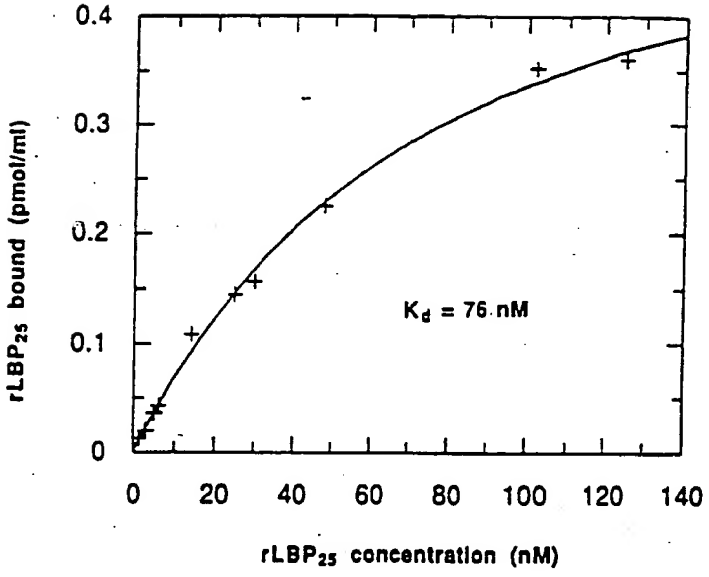

0.4
0.3
0.2
0.1
0
rLBP25 bound (pmol/ml)
Kd = 76 nM
0
20
40
60
80
100
120
140
rLBP25 concentration (nM)

FIGURE 9

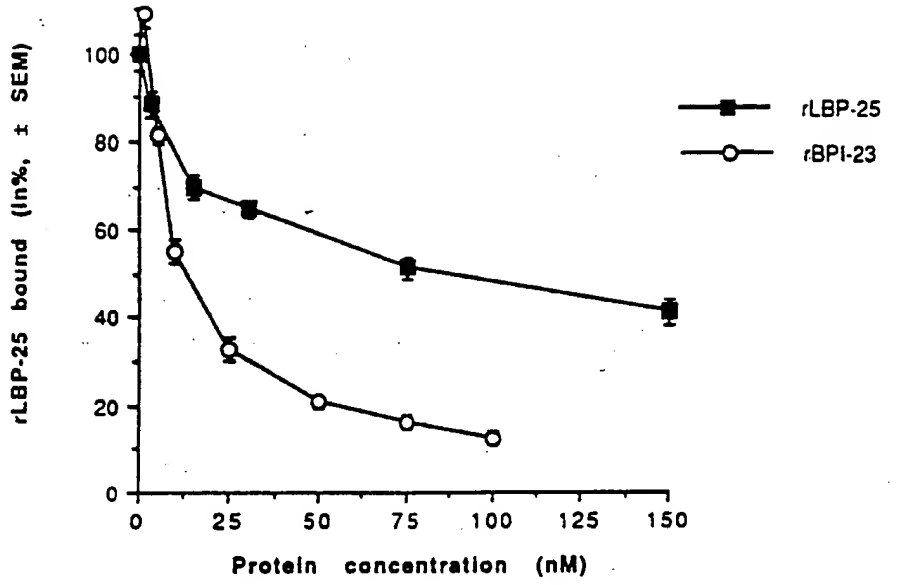


FIGURE 10

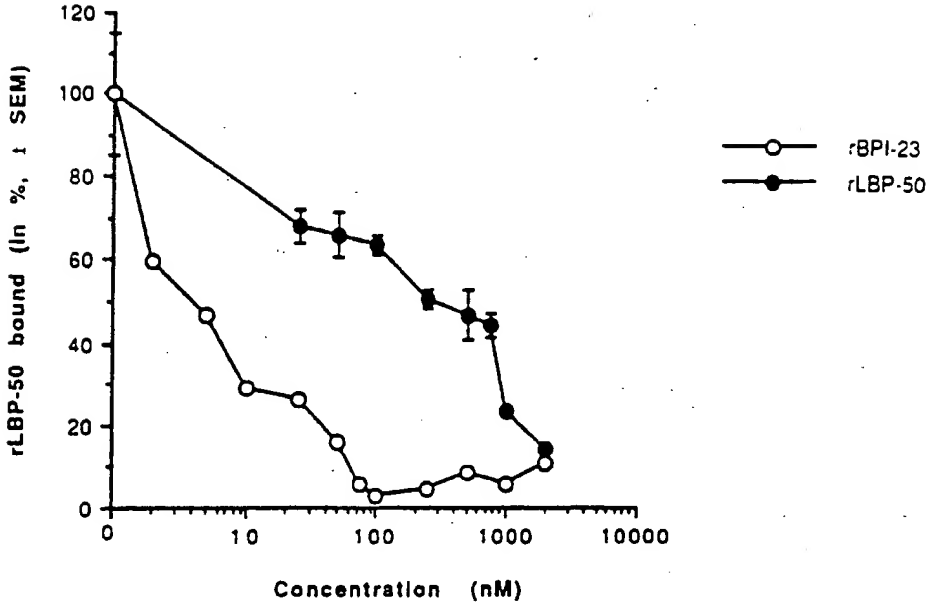


FIGURE 11

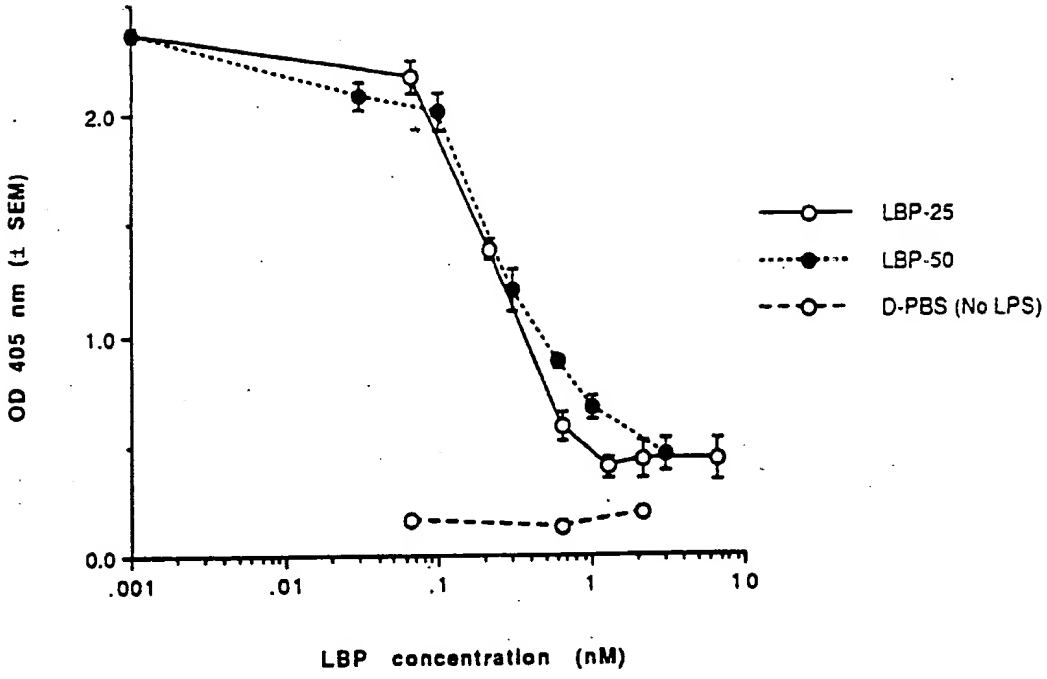

OD 405 nm (± SEM)
2.0
1.0
0.0
.001
.01
.1
1
10
LBP concentration (nM)
LBP-25
LBP-50
D-PBS (No LPS)

FIGURE 12

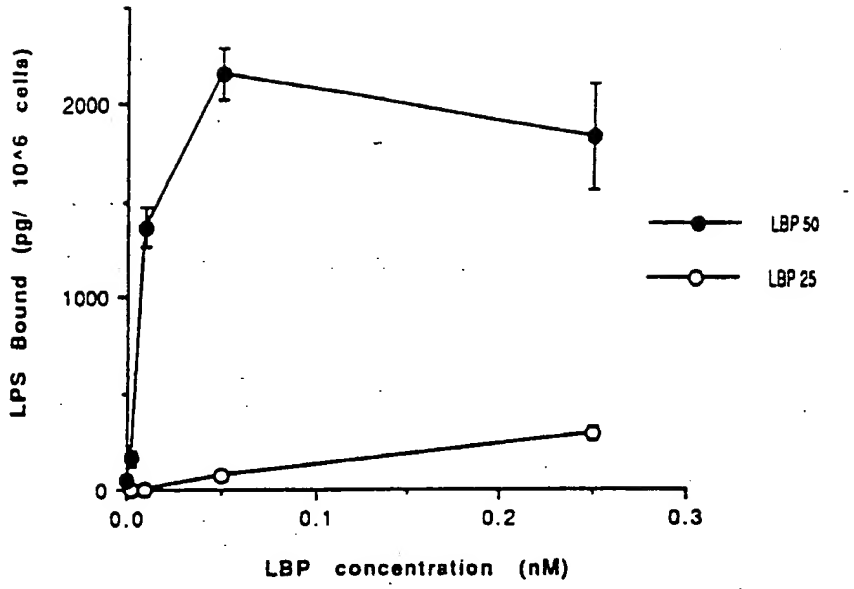


FIGURE 13

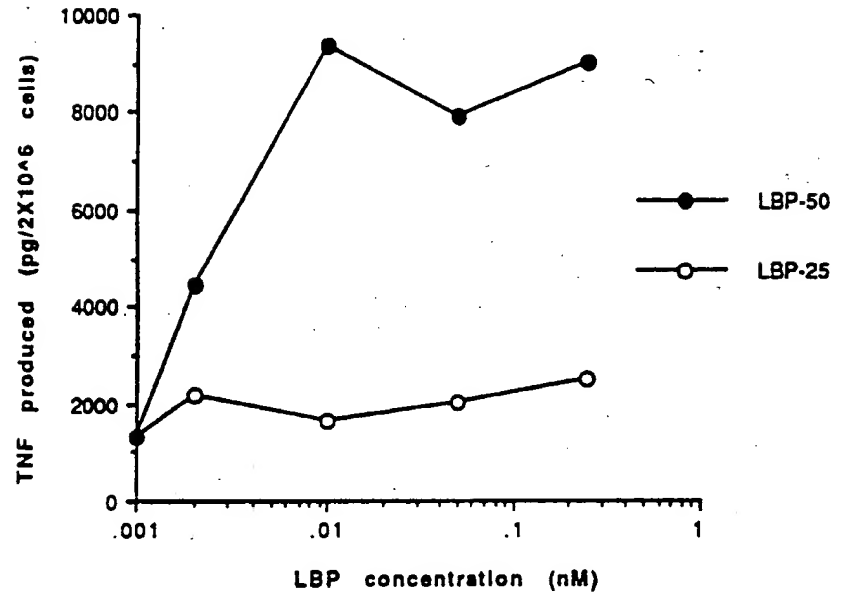


FIGURE 14

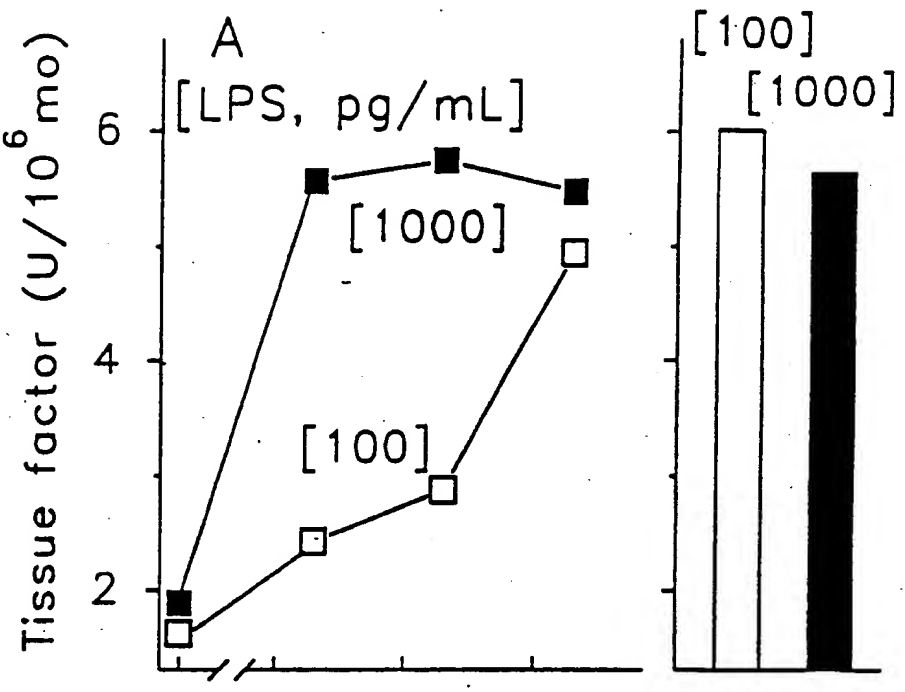


FIGURE 15A

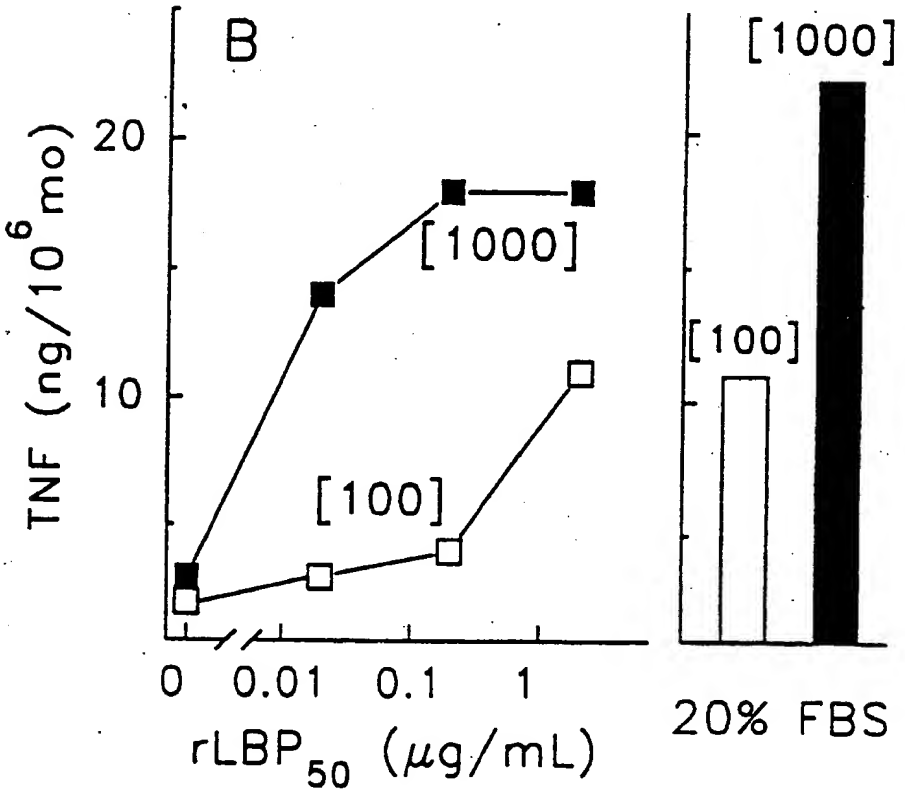


FIGURE 15B

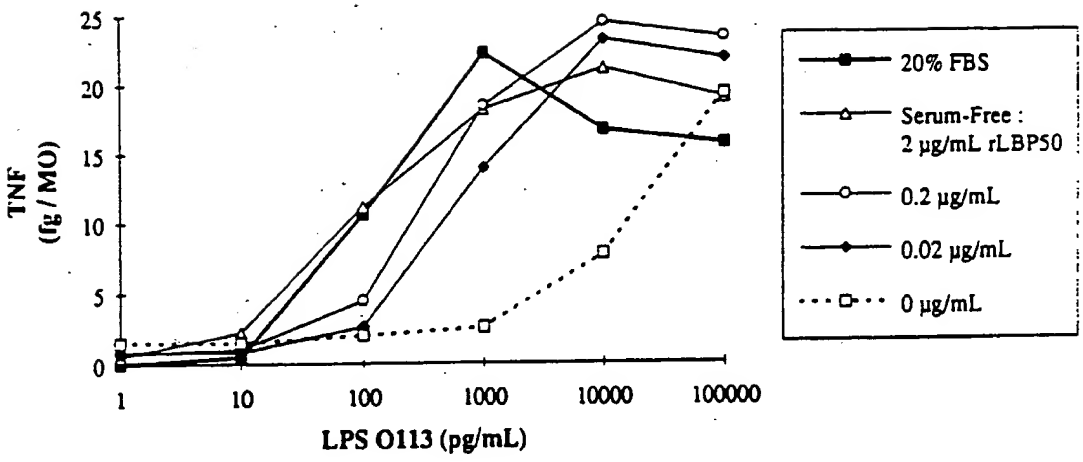

TNF
(fg / MO)
25
20
15
10
5
0
1
10
100
1000
10000
100000
LPS O113 (pg/mL)
20% FBS
Serum-Free :
2 µg/mL rLBP50
0.2 µg/mL
0.02 µg/mL
0 µg/mL

FIGURE 16

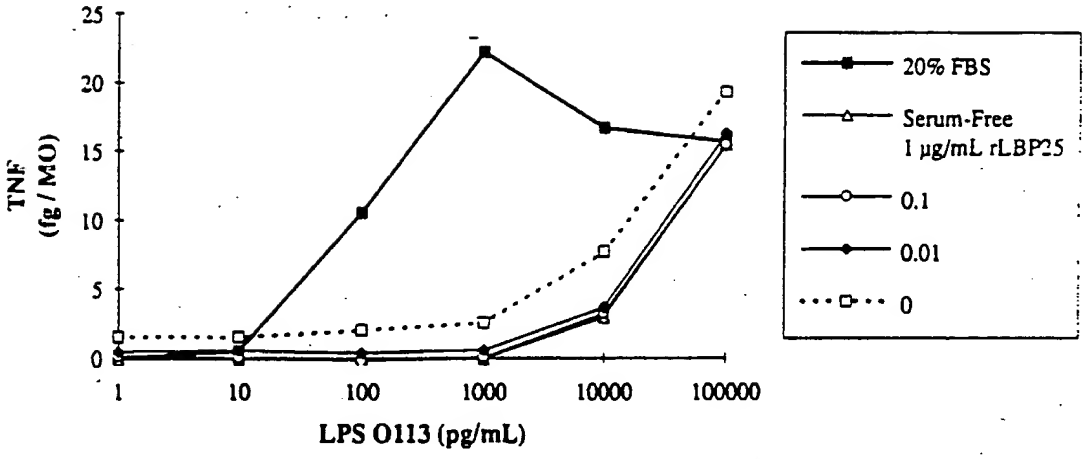

TNF
(fg / MO)
25
20
15
10
5
0
1
10
100
1000
10000
100000
LPS O113 (pg/mL)
20% FBS
Serum-Free
1 µg/mL rLBP25
0.1
0.01
0

FIGURE 17

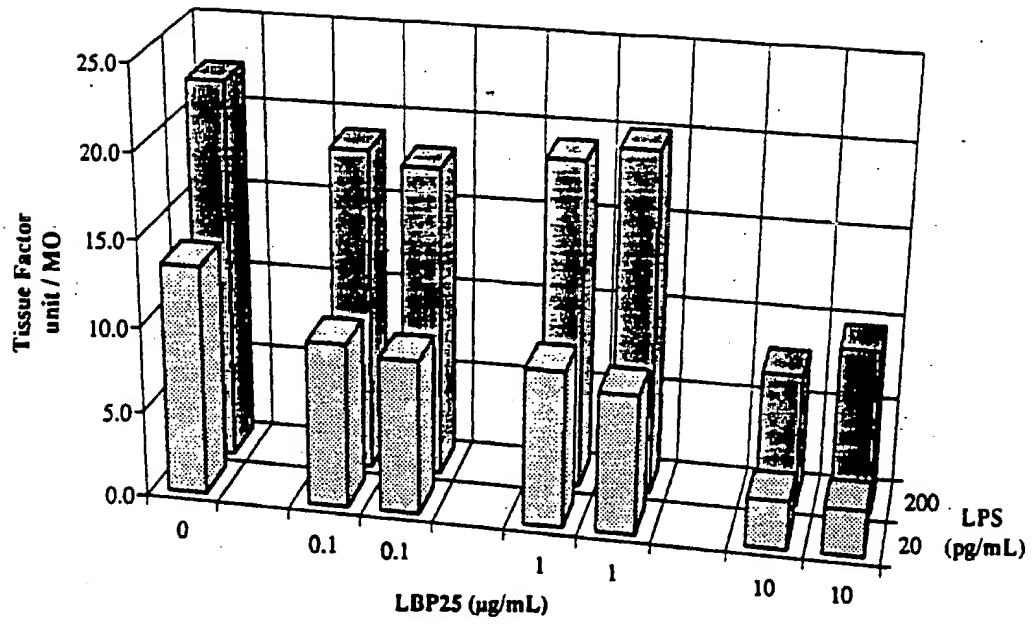

Tissue Factor unit / MO
25.0
20.0
15.0
10.0
5.0
0.0
0
0.1
0.1
1
1
10
10
LBP25 (µg/mL)
200
20
LPS (pg/mL)

FIGURE 18

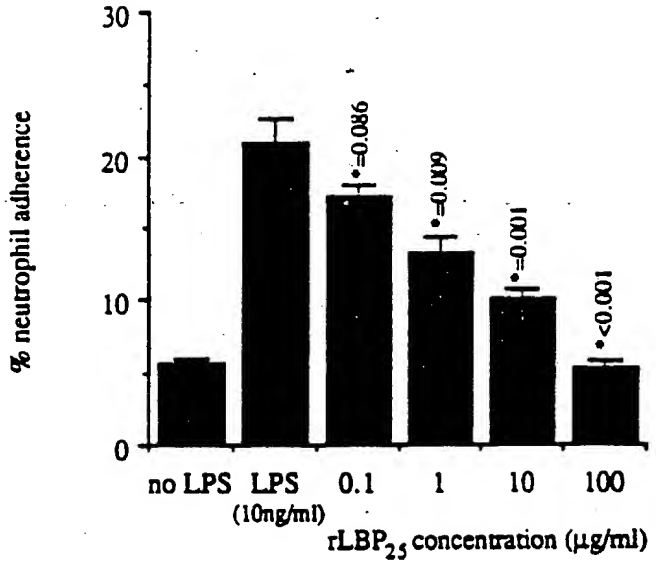

30
20
10
0
% neutrophil adherence
*=0.086
*=0.009
*=0.001
*<0.001
no LPS
LPS (10ng/ml)
0.1
1
10
100
rLBP25 concentration (µg/ml)

FIGURE 19

FIGURE 20

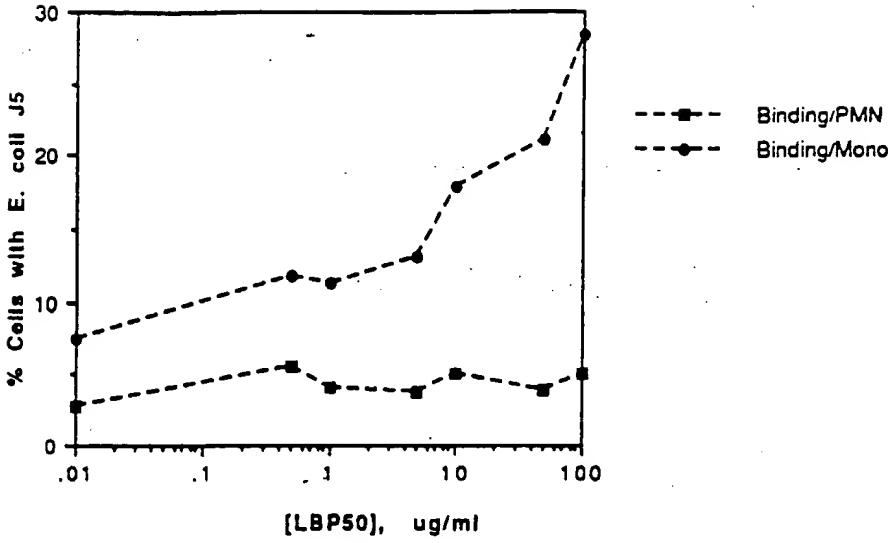


FIGURE 21

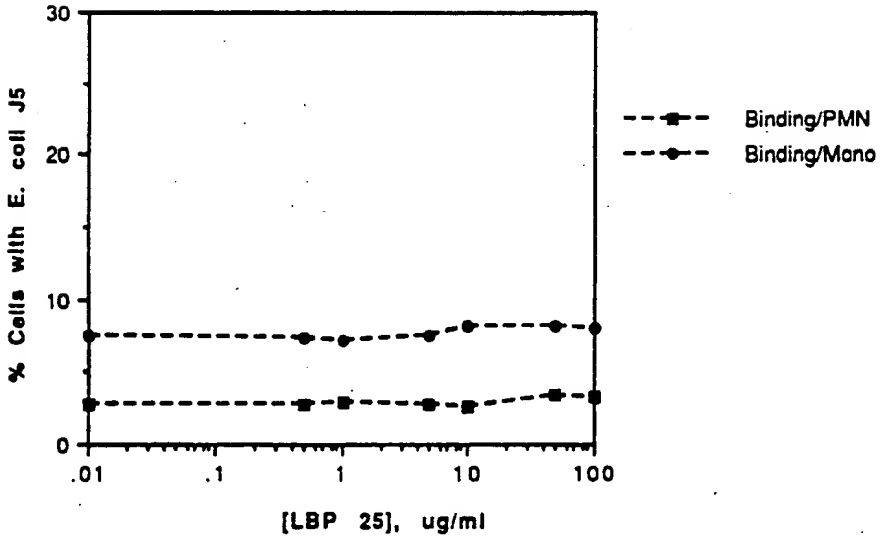
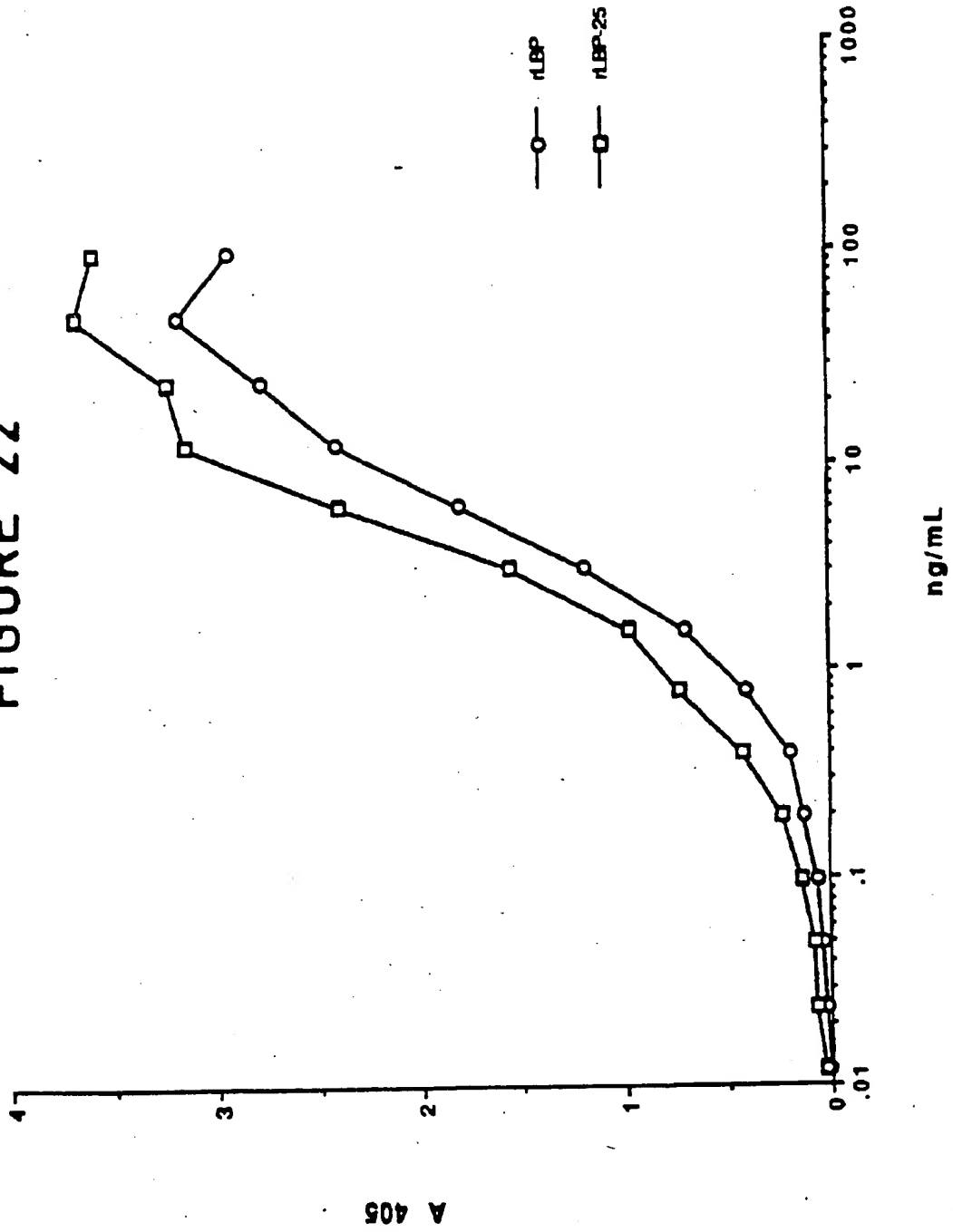


FIGURE 22



BPI_{23}/LBP_{25} SEQUENCE ALIGNMENT

```
              10        20        30        40        50        60
BPI...VNPGVVVRISQKGLDYASQQGTAALQKELKRIKIPDYSDSFKIKHLGKGHVSFYSMDIRE
       ::: : ::  ::: :: : :  ::: :: ::  ::      : : : :   : :  :
LBP...ANPGLVARITDKGLQYAAQEGLLALQSELLRITLPDFTGDLRIPHVGRGRYEFHSLNIHS
              10        20        30        40        50        60

              70        80        90       100       110       120
BPI...FQLPSSQISMVPNVGLKFSISNANIKISGKWKAQKRFLKMSGNFDLSIEGMSISADLKLG
        :  :    ::  ::  :::   :   : ::  : : :  : :: :  : :::  : ::
LBP...CELLHSALRPVPGQGLSLSISDSSIRVQGRWKVRKSFFKLQGSFDVSVKGISISVNLLLG
              70        80        90       100       110       120

             130       140       150       160       170       180
BPI...SNPTSGKPTITCSSCSSHINSVHVHISKSKVGWLIOLFHKKIESALRNKMNSQVCEKVTN
      :   :: :: : ::::: :  : :  :    :::  :::  :::       :  ::
LBP...SE-SSGRPTVTASSCSSDIADVEVDMSG-DLGWLLNLFHNQIESKFQKVLESRICEMIQK
             130       140        150       160       170

             190      199
BPI...SVSSELQPYFQTLPVMTKI
      :::: :::: ::::: : :
LBP...SVSSDLQPYLQTLPVTTEI
     180       190     197
```

FIGURE 23

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 94/06931

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/12 C07K13/00 C12N15/62 A61K37/02 C12P21/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C07K C12N C12P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 03535 (INCYTE PHARMACEUTICALS, INC.) 5 March 1992 cited in the application see example 5	1-3
A	see figure 23	1-17

A	WO,A,92 09621 (NEW YORK UNIVERSITY) 11 June 1992 see page 8, line 19 - page 10, line 33 see examples 3,4	6-9

P,X	JOURNAL OF IMMUNOLOGY 152 (7). 1994. 3623-3629 THEOFAN, G. ET AL. 'An amino-terminal fragment of human lipopolysaccharide -binding protein retains lipid A binding but not CD14-stimulatory activity.' see the whole document	1-4

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 October 1994	21. 10. 94

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Andres, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 94/06931

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO,A,93 23540 (XOMA CORPORATION) 25 November 1993 see examples 3,4	1-4

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

.ernational application No.

PCT/US 94/06931

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 15-16 are directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

...information on patent family members

Inter nal Application No
PCT/US 94/06931

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9203535	05-03-92	US-A-	5171739	15-12-92
		AU-A-	8850191	17-03-92
		EP-A-	0544832	09-06-93
		JP-T-	6504267	19-05-94
		US-A-	5234912	10-08-93
		US-A-	5334584	02-08-94
		US-A-	5308834	03-05-94
WO-A-9209621	11-06-92	AU-A-	9127591	25-06-92
		EP-A-	0563222	06-10-93
WO-A-9323540	25-11-93	AU-B-	4382093	13-12-93